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DATE: Tuesday, May 10, 2005

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		DB=USPT; PLUR=YES; OP=AND	
☐	L1	methylase.clm. or methyltransferase.clm. or dam$2.clm.	6090
☐	L2	L1 and gatc	31
☐	L3	L1 and 2$gatc$2	0
☐	L4	L1 and $gatc$	57
☐	L5	dam near5 (methylase or methyltransferase)	185
☐	L6	L5 not l2	163

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 6872547 B1
TITLE: Functional balanced-lethal host-vector systems

Brief Summary Text (31):
In additional embodiments, the present invention is directed to a method of delivering a desired gene product to a vertebrate. The method comprises administering to the vertebrate live bacterial cells of an attenuated derivative of a pathogenic bacterium. The bacterial cells are as described above. In these embodiments the preferred bacteria are Salmonella, most preferably S. typhimurium, S. typhi, S. paratyphi, S. choleraesuis, S. dublin or S. gallinarum. As before, preferred attenuating mutations are in a pab gene, a pur gene, an aro gene, asd, a dap gene, nadA, pncB, galE, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, dam, phoP, phoQ, rfc, poxA, galU, mviA, sodC, recA, ssrA, sirA, inv, hiLA, rpoE, flgM, tonB, slyA, and combinations thereof, and the most preferred essential gene is an asd. Preferred desired gene products in these embodiments include antigens, lymphokines, cytokines, and sperm-specific or egg-specific autoantigens.

L3: Entry 4 of 19 File: USPT Jul 1, 2003

DOCUMENT-IDENTIFIER: US 6585975 B1
TITLE: Use of Salmonella vectors for vaccination against helicobacter infection

Detailed Description Text (4):
Numerous attenuated Salmonella vectors that can be used in the invention are known in the art, and can be derived from species such as, for example, S. typhi, S. typhimurium, S. enteritidis, S. dublin, S. Minnesota, and S. choleraesuis. The vectors can be attenuated chemically (e.g., Ty21a, Swiss Serums and Vaccines, Berna Products) or, preferably, by genetic mutagenesis (e.g., Ty800). For example, attenuation can be achieved by inactivation of key regulatory genes or genes necessary for in vivo survival. For example, the following genes can be inactivated: cya, crp, and asd (cAMP metabolism; see, e.g., Curtiss et al., Vaccine 6:155-160, 1988; Nakayama et al., BioTechnology 6:693, 1988; WO 92/11361), adenylate cyclase and the cAMP receptor (U.S. Pat. No. 5,389,368), cdt (invasion of liver and spleen), phoP/phoQ (two component regulator; see, e.g., Fields et al., Science 243:1059-1062, 1989; U.S. Pat. No. 5,424,065), ompR (control of capsule and porin expression; see, e.g., Dorman et al., Infection and Immunity 57:2136-2140, 1989), outer membrane proteins (U.S. Pat. No. 5,527,529), reverse mutants of streptomycin mutants (U.S. Pat. No. 4,350,684), genes in pathogenicity islands (Shea et al., Infection and Immunity 67:213-219, 1999; WO 99/37759), SPI-2 (invasion of Peyer's patches), Dam (DNA methylation), htrA (heat shock protein; U.S. Pat. No. 5,804,194), and other heat shock proteins (U.S. Pat. No. 5,804,194). The vectors can also be attenuated by auxotrophic mutations, such as mutations in any of the aroA, aroC, aroD (aromatic compounds), purA, or guaAB (purines) genes (see, e.g., U.S. Pat. No. 5,770,214).

CLAIMS:

1. A method of inducing an immune response against Helicobacter in a mammal, said method comprising the steps of: mucosally administering to said mammal an attenuated Salmonella vector comprising a nucleic acid molecule encoding a Helicobacter antigen, and parenterally administering to said mammal a Helicobacter antigen.

2. The method of claim 1, wherein said attenuated Salmonella vector is administered orally to said mammal.

L3: Entry 9 of 19 File: USPT Feb 26, 2002

DOCUMENT-IDENTIFIER: US 6350454 B1
TITLE: Attenuated Pasteurella piscicida vaccine for fish

Detailed Description Text (62):
Briefly, the same techniques as described above will be used to create aroA deletion mutants or purA deletion mutants, where the inserted sequences contain both the kanamycin resistance gene to facilitate selection, and also a gene encoding the heterologous antigen. Preferably the gene for the heterologous antigen is placed under the control of the native promoter for the aroA gene or pura gene, as appropriate, to ensure that the antigen is expressed and is 'seen" by the fish immune system during the relatively brief residence of the attenuated strain in the fish before it is cleared. The aroA promoter will be active in conditions where the attenuated E. ictaluri is starved for aromatic amino acids; and the purA promoter will be active in conditions where the attenuated E. ictaluri is starved for adenine. Alternatively, the gene for the heterologous antigen may be placed under the control of a constitutive promoter, such as the constitutive E. ictaluri methylase gene promoter. See Jie Zhang, "Identification, Cloning and Sequence of a Methylase Gene from Edwardsiella ictaluri," M. S. Thesis (Louisiana State University, Baton Rouge, 1995). The E. coli alkaline phosphatase promoter is also known to be constitutive in E. ictaluri.

CLAIMS:

11. A method of reducing the susceptibility of a fish to infection by a pathogen that expresses the antigenic peptide or antigenic protein that is encoded by the exogenous gene of the attenuated Pasteurella piscicida bacterium comprised in the vaccine of claim 10, comprising administering to the fish a protective amount of said vaccine.

: Entry 9 of 19 File: USPT Feb 26, 2002

US-PAT-NO: 6350454
DOCUMENT-IDENTIFIER: US 6350454 B1

TITLE: Attenuated Pasteurella piscicida vaccine for fish

DATE-ISSUED: February 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thune; Ronald L.	Baton Rouge	LA		

US-CL-CURRENT: 424/200.1; 424/184.1, 424/201.1, 424/203.1, 424/234.1, 424/235.1, 424/255.1, 424/827, 424/93.4

CLAIMS:

What is claimed:

1. A vaccine comprising a protective amount of the attenuated Pasteurella piscicida bacterium with ATCC accession number 202110.

2. A vaccine comprising a protective amount of an attenuated Pasteurella piscicida bacterium, wherein said attenuated Pasteurella piscicida bacterium comprises the artificial aroA gene mutation as found in the bacterium with ATCC accession number 202110.

3. The vaccine as recited-in claim 1, wherein said bacterium is the progeny of the bacterium with ATCC accession number 202110; wherein said bacterium has the aromatic amino acid auxotrophic characteristics of the bacterium with ATCC accession number 202110.

4. The vaccine as recited in claim 1, wherein said bacterium additionally comprises an exogenous gene encoding an antigenic peptide or antigenic protein that is native to a fish pathogen other than Pasteurella piscicida.

5. A method of reducing the susceptibility of a fish to Pasteurellosis, comprising administering to the fish the vaccine as recited in claim 2.

6. The method as recited in claim 5, wherein the fish is selected from the group consisting of percichthyids, serranids, carangids, balistids, and sparids.

7. The method as recited in claim 5, wherein said administering step comprises immersing the fish in said vaccine.

8. The method as recited in claim 5, wherein said administering step comprises feeding the fish a food product comprising said vaccine.

9. The method as recited in claim 5, wherein said administering step comprises injecting the fish with said vaccine intraperitoneally.

10. The vaccine as recited in claim 2, wherein said attenuated Pasteurella piscicida bacterium additionally comprises an exogenous gene encoding an antigenic peptide or antigenic protein that is native to a fish pathogen other than Pasteurella piscicida.

11. A method of reducing the susceptibility of a fish to infection by a pathogen that expresses the antigenic peptide or antigenic protein that is encoded by the exogenous gene of the attenuated Pasteurella piscicida bacterium comprised in the vaccine of claim 10, comprising administering to the fish a protective amount of said vaccine.

12. The method as recited in claim 11, wherein the fish is selected from the group consisting of percichthyids, serranids, carangids, balistids, and sparids.

13. The method as recited in claim 11, wherein said administering step comprises immersing the fish in said vaccine.

14. The method as recited in claim 11, wherein said administering step comprises feeding the fish a food product comprising said vaccine.

15. The method as recited in claim 11, wherein said administering step comprises injecting the fish with said vaccine intraperitoneally.

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☐	L1	(aroA or aro-A) same dam	3
		DB=EPAB,JPAB,DWPI; PLUR=YES; OP=AND	
☐	L2	9937759	3
☐	L3	salmonell$.ti. and attenuat$.ti.	44
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L4	(salmonell$.ti. and attenuat$.ti.) and (pura or pur-a)	3

END OF SEARCH HISTORY

6585975
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lines 62-63
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Hide?	Set Name	Query	Hit Count
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☐	L1	attenuat$ or avirulent or a-virulent or weakened or mutated or mutant or noninvasive or non-invasive or altered	357118
☐	L2	L1.clm. same administer$.clm.	483
☐	L3	L2 and (dam or gatc or methylase or methyltransferase or methyl-transferase)	19
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND		
☐	L4	L2 and (dam or gatc or methylase or methyltransferase or methyl-transferase)	19
☐	L5	attenuat$ or avirulent or a-virulent or weakened or mutated or mutant or noninvasive or non-invasive or altered	773166
☐	L6	L5 near10 administer$	5691
☐	L7	L6 same (dam or gatc or methylase or methyltransferase or methyl-transferase)	10
☐	L8	L7 not l4	10

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☐	L1	attenuat$ or avirulent or a-virulent or weakened or mutated or mutant or noninvasive or non-invasive or altered	357118
☐	L2	L1.clm. same administer$.clm.	483
☐	L3	L2 and (dam or gatc or methylase or methyltransferase or methyl-transferase)	19

END OF SEARCH HISTORY

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2. 20020086032. 09 Aug 01. 04 Jul 02. Producing antibodies with attenuated bacteria with altered DNA adenine methylase activity. Mahan, Michael J., et al. 424/200.1; 435/252.3 A61K039/02 C12N001/21.

3. 20020081317. 09 Aug 01. 27 Jun 02. Bacteria with altered DNA adenine methylase (DAM) activity and heterologous epitope. Mahan, Michael J., et al. 424/200.1; 435/252.3 435/320.1 A61K039/02 C12N001/21 C12N015/74.

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6. 20020068068. 09 Aug 01. 06 Jun 02. Method of creating antibodies and compositions used for same. Mahan, Michael J., et al. 424/200.1; 424/257.1 424/258.1 424/261.1 A61K039/108 A61K039/112 A61K039/106 A61K039/02.

7. 6872547. 11 Oct 00; 29 Mar 05. Functional balanced-lethal host-vector systems. Curtiss, III; Roy. 435/69.1; 424/257.1 424/258.1 435/252.1 435/252.3 435/471. C12P02106 C12N00112 C12N00120 A61K039108 A61K039112.

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9. 3911108. 14 Feb 73; 07 Oct 75. Process of producing bovine milk products containing specific antibodies. Singh, deceased; Vipin K.. 424/157.1; 424/223.1 426/335. A61K023/00.

10. WO 200045840A. New vaccine compositions comprising pathogenic bacteria which contain a mutation affecting DNA adenine methylase, useful for treating or preventing bacterial infection, especially Salmonella infection, in humans and domestic animals. HEITHOFF, D M, et al. A61K000/00 A61K039/00 A61K039/02 A61K039/095 A61K039/10 A61K039/102 A61K039/106 A61K039/108 A61K039/112 A61K048/00 A61P031/04 A61P037/00 C12N001/20 C12N001/21 C12N001/36 C12N001:20 C12N015/74 C12R001/42 C12R001:42 G01N033/02 G01N033/15 G01N033/50 G01N033/53 G01N033/566 C12N001/20 C12R001:42.

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		DB=USPT; PLUR=YES; OP=AND	
☐	L1	thune.in.	28
☐	L2	L1 and salmonella	2
☐	L3	salmonella.clm.	903
☐	L4	L3 and arob.clm.	1
☐	L5	L3 and aro-b.clm.	0
☐	L6	L3 and aro.clm.	19
☐	L7	cysg	25
☐	L8	cysg and (dam or arob or aro-b)	5

END OF SEARCH HISTORY

Mol Gen Genet. 1995 Jun 10;247(5):546-54. Related Articles, Links

Characterization of three genes in the dam-containing operon of Escherichia coli.

Lyngstadaas A, Lobner-Olesen A, Boye E.

Department of Biophysics, Institute for Cancer Research, Montebello, Oslo, Norway.

The dam-containing operon in Escherichia coli is located at 74 min on the chromosomal map and contains the genes aroK, aroB, a gene called urf74.3, dam and trpS. We have determined the nucleotide sequence between the dam and trpS genes and show that it encodes two proteins with molecular weights of 24 and 27 kDa. Furthermore, we characterize the three genes urf74.3, 24kDa, 27kDa and the proteins they encode. The predicted amino acid sequences of the 24 and 27 kDa proteins are similar to those of the CbbE and CbbZ proteins, respectively, of the Alcaligenes eutrophus cbb operon, which encodes enzymes involved in the Calvin cycle. In separate experiments, we have shown that the 24 kDa protein has d-ribulose-5-phosphate epimerase activity (similar to CbbE), and we call the gene rpe. Similarly, the 27 kDa protein has 2-phosphoglycolate phosphatase activity (similar to CbbZ), and we name the gene gph. The Urf74.3 protein, with a predicted molecular weight of 46 kDa, migrated as a 70 kDa product under denaturing conditions. Overexpression of Urf74.3 induced cell filamentation, indicating that Urf74.3 directly or indirectly interferes with cell division. We present evidence for translational coupling between aroB and urf74.3 and also between rpe and gph. Proteins encoded in the dam superoperon appear to be largely unrelated: Dam, and perhaps Urf74.3, are involved in cell cycle regulation, AroK, AroB, and TrpS function in aromatic amino acid biosynthesis, whereas Rpe and Gph are involved in carbohydrate metabolism.

PMID: 7603433 [PubMed - indexed for MEDLINE]

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ClinicalTrials.gov
PubMed Central

1: Mol Gen Genet. 1989 May;217(1):85-96. Related Articles, Links

The Escherichia coli dam gene is expressed as a distal gene of a new operon.

Jonczyk P, Hines R, Smith DW.

Department of Biology, University of California, San Diego, La Jolla 92093.

DNA containing the Escherichia coli dam gene and sequences upstream from this gene were cloned from the Clarke-Carbon plasmids pLC29-47 and pLC13-42. Promoter activity was localized using pKO expression vectors and galactokinase assays to two regions, one 1650-2100 bp and the other beyond 2400 bp upstream of the dam gene. No promoter activity was detected immediately in front of this gene; plasmid pDam118, from which the nucleotide sequence of the dam gene was determined, is shown to contain the pBR322 promoter for the primer RNA from the pBR322 rep region present on a 76 bp Sau3A fragment inserted upstream of the dam gene in the correct orientation for dam expression. The nucleotide sequence upstream of dam has been determined. An open reading frame (ORF) is present between the nearest promoter region and the dam gene. Codon usage and base frequency analysis indicate that this is expressed as a protein of predicted size 46 kDa. A protein of size close to 46 kDa is expressed from this region, detected using minicell analysis. No function has been determined for this protein, and no significant homology exist between it and sequences in the PIR protein or GenBank DNA databases. This unidentified reading frame (URF) is termed urf-74.3, since it is an URF located at 74.3 min on the E. coli chromosome. Sequence comparisons between the regions upstream of urf-74.3 and the aroB gene show that the aroB gene is located immediately upstream of urf-74.3, and that the promoter activity nearest to dam is found within the aroB structural gene. This activity is relatively weak (about 15% of that of the E. coli gal operon promoter). The promoter activity detected beyond 2400 bp upstream of dam is likely to be that of the aroB gene, and is 3 to 4 times stronger than that found within the aroB gene. Three potential DnaA binding sites, each with homology of 8 of 9 bp, are present, two in the aroB promoter region and one just upstream of the dam gene. Expression through the site adjacent to the

dam gene is enhanced 2- to 4-fold in dnaA mutants at 38 degrees C. Restriction site comparisons map these regions precisely on the Clarke-Carbon plasmids pLC13-42 and pLC29-47, and show that the E. coli ponA (mrcA) gene resides about 6 kb upstream of aroB.

PMID: 2549371 [PubMed - indexed for MEDLINE]

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Mol Microbiol. 1994 May;12(4):631-8. Related Articles, Links

Novel growth rate control of dam gene expression in Escherichia coli.

Rasmussen LJ, Marinus MG, Lobner-Olesen A.

Department of Pharmacology, University of Massachusetts Medical School, Worcester 01655.

Transcription of the dam gene in Escherichia coli is growth rate regulated by a mechanism distinct from that used for ribosomal RNA gene promoters. Single-copy operon fusions to lacZ indicated that the major promoter, P2, is responsible for most or all of the growth rate dependence. Promoter P2 is a typical sigma 70 promoter with 18 bp spacing between the -10 and -35 hexamers. Primer extension analysis was used to show that there was no inhibition of transcription from promoter P2 in cells induced for the stringent response. Beta-galactosidase specific activity from a single-copy dam::lacZ fusion was unaffected by either excess rrnB RNA or the level of Fis protein. Thus growth rate control of dam gene expression differs from that of the rRNA and tRNA genes by its lack of response to stringent control, ribosomal feedback and enhanced transcription by Fis protein. We devised a procedure for selection of mutant cells in which dam gene expression was unregulated. One such mutant (cde-4), obtained by miniTn10 insertion, showed the same level of beta-galactosidase activity at all growth rates tested. In contrast, growth rate-dependent expression of the rrnB gene was unaffected by cde-4 confirming the different modes of regulation. The cde-4::miniTn10 insertion is located close to kilobase 670 on the physical map in or near the lipB gene.

PMID: 7934887 [PubMed - indexed for MEDLINE]

Gene. 1982 Dec;20(2):197-204. Related Articles, Links

Cloning of the modification methylase gene of Bacillus centrosporus in Escherichia coli.

Janulaitis A, Povilionis P, Sasnauskas K.

The gene specifying a sequence-specific modification methylase of Bacillus centrosporus has been cloned in Escherichia coli using the restriction endonuclease HindIII and the plasmid pBR322. The selection was based on detection of new methylation properties rendering recombinant plasmids carrying the methylase gene nonsusceptible to BcnI endonuclease cleavage. The presence of a 3.2-kb HindIII fragment in either orientation conferred BcnI resistance on the recombinant plasmids. These results suggest that the BcnI methylase gene is expressed in E. coli under the control of a promoter located on the cloned fragment. The relative level of BcnI methylase enzyme in E. coli was similar to that in B. centrosporus. The recombinant clones do not exhibit any BcnI restriction-endonuclease activity.

PMID: 6299887 [PubMed - indexed for MEDLINE]

US-PAT-NO: 6680182
DOCUMENT-IDENTIFIER: US 6680182 B1
**** See image for Certificate of Correction ****

TITLE: Expression of recombinant fusion proteins in attenuated bacteria

DATE-ISSUED: January 20, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Khan; Mohammed Anjam	Cambridge			GB
Villarreal-Ramos; Bernardo	Cambridge			GB
Hormaeche; Carlos Estenio	Newcastle upon Tyne			GB
Chatfield; Steven Neville	London			GB
Dougan; Gordon	London			GB

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Acambis Research Limited	Slough			GB	03

APPL-NO: 08/ 379611 [PALM]
DATE FILED: July 21, 1995

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9216317	July 31, 1992
GB	9306398	March 26, 1993

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/GB93/01617	July 30, 1993	WO94/03615	Feb 17, 1994		

INT-CL: [07] C12 N 15/09

US-CL-ISSUED: 435/69.7; 435/69.3, 424/200.1, 424/258.1, 424/93.2, 424/191.1
US-CL-CURRENT: 435/69.7; 424/191.1, 424/200.1, 424/258.1, 424/93.2, 435/69.3

FIELD-OF-SEARCH: 424/200.1, 424/258.1, 424/93.2, 424/191.1, 435/69.3, 435/69.7

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
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☐	5389540	February 1995	Makoff et al.	435/69.3
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ART-UNIT: 1645

PRIMARY-EXAMINER: Smith; Lynette R. F.

ASSISTANT-EXAMINER: Portner; Ginny Allen

ATTY-AGENT-FIRM: Wolf, Greenfield & Sacks, P.C.

ABSTRACT:

The invention provides a DNA molecule comprising a promoter sequence operably linked to a DNA sequence encoding first and second proteins linked by a hinge region wherein in that the promoter sequence can be one having activity which is induced in response to a change in the surrounding environment and the first protein can be Tetanus toxin C fragment or one or more epitopes thereof. The invention also provides intermediate molecules having a promoter operably linked to a DNA sequence encoding a first antigenic sequence and a hinge region, and at or adjacent the 3'-end thereof one or more restriction sites for the introduction of a second anti-genic sequence. In addition, the invention provides replicable expression vectors containing the DNA fusion proteins expressed therefrom, bacterial transformed with the vectors and the use of the bacteria, in vaccines.

15 Claims, 18 Drawing figures

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L3: Entry 6 of 13 File: USPT Sep 19, 2000

DOCUMENT-IDENTIFIER: US 6120774 A
TITLE: Heterophil-adapted poultry vaccine

CLAIMS:

21. The vaccine of claim 11, wherein said poultry heterophil-adapted strain is in the form of a culture, frozen sample, lyophilized sample, or agar stab.

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DOCUMENT-IDENTIFIER: US 6231871 B1
TITLE: Live in ovo vaccine

CLAIMS:

1. A method for immunizing an avian species against infection by a pathogenic Salmonella microorganism, said method comprising administering in ovo to an air sac of an egg an attenuated Salmonella microorganism which:

(a) exhibits auxotrophy to one or more growth factors selected from the group consisting of phenylalanine, tyrosine, tryptophan and para-aminobenzoic acid such that it is incapable of growing on a minimal medium in the absence of said one or more growth factors; and

(b) is capable of colonizing one or more tissues in an embryo prior to hatching in an amount and under conditions effective to induce an immune response in the embryo before or immediately after hatching against a virulent form of said attenuated Salmonella microorganism or a microorganism expressing an immunologically cross-reactive antigen.

4. A method according to claim 1 wherein the attenuated Salmonella microorganism is a Salmonella selected from the group consisting of Salmonella typhimurium, Salmonella paratyphi A or C, Salmonella schottmulleri, Salmonella choleraesuis, Salmonella montevideo, Salmonella newport, Salmonella enteritidis, Salmonella gallinarum, Salmonella pullorum, Salmonella abortusovi, Salmonella abortus-equi, Salmonella dublin, Salmonella sofia, Salmonella havana, Salmonella bovismorbificians, Salmonella hadar, Salmonella arizonae and Salmonella anatum.

5. A method according to claim 4 wherein the attenuated Salmonella microorganism is Salmonella typhimurium.

6. A method according to claim 5 wherein the attenuated Salmonella microorganism is Salmonella typhimurium strain STM-1, deposited at the Australian Government Analytical Laboratories under Accession number N93/43266.

7. A method according to claim 1 wherein the attenuated Salmonella microorganism is incapable of synthesizing chorismate such that the Salmonella microorganism is incapable of growing on minimal medium.

8. A method according to claim 7 wherein the attenuated Salmonella microorganism carries a nucleotide substitution, deletion, insertion, or combination thereof in one or more genes selected from the group consisting of aroA, aroB, aroC and aroD.

9. A method according to claim 8 wherein the attenuated Salmonella microorganism carries a deletion in at least one gene selected from the group consisting of aroA, aroB, aroC and aroD.

10. A method according to claim 1 wherein the attenuated Salmonella microorganism breaks through the air sac and the embryo is exposed to the vaccine.

11. A method according to claim 1 wherein the attenuated Salmonella microorganism competitively excludes pathogenic microorganisms from a newly-hatched bird.

12. Salmonella typhimurium STM-1 deposited at the Australian Government Analytical Laboratories

under Accession number N93/43266.

13. A fertilized egg from an avian species having an air sac wherein the air sac is inoculated with an attenuated Salmonella microorganism which:

(a) exhibits auxotrophy to one or more growth factors selected from the group consisting of phenylalanine, tyrosine, tryptophan and para-aminobenzoic acid such that it is incapable of growing on a minimal medium in the absence of said one or more growth factors;

(b) is capable of colonizing one or more tissues in an embryo prior to hatching; and

(c) is capable of inducing before or immediately after hatching an immune response in the embryo against a virulent form of said Salmonella microorganism or a microorganism expressing an immunologically cross-reactive antigen.

16. A fertilized egg according to claim 13 wherein the attenuated Salmonella microorganism is a Salmonella selected from the group consisting of Salmonella typhimurium, Salmonella paratyphi A or C, Salmonella schottmulleri, Salmonella choleraesuis, Salmonella montevideo, Salmonella newport, Salmonella enteritidis, Salmonella gallinarum, Salmonella pullorum, Salmonella abortusovi, Salmonella abortus-equi, Salmonella dublin, Salmonella sofia, Salmonella havana, Salmonella bovismorbificians, Salmonella hadar, Salmonella arizonae and Salmonella anatum.

17. A fertilized egg according to claim 16 wherein the attenuated Salmonella microorganism is Salmonella typhimurium or Salmonella enteritidis.

18. A fertilized egg according to claim 17 wherein the attenuated Salmonella microorganism is Salmonella typhimurium strain STM-1, deposited at the Australian Government Analytical Laboratories under Accession number N93/43266.

19. A fertilized egg according to claim 13 wherein the attenuated Salmonella microorganism is incapable of synthesizing chorismate such that the microorganism is incapable of growing on minimal medium.

20. A fertilized egg according to claim 19 wherein the attenuated Salmonella microorganism carries a nucleotide substitution, deletion, insertion, or combination thereof in one or more genes selected from the group consisting of aroA, aroB, aroC and aroD.

21. A fertilized egg according to claim 20 wherein the attenuated Salmonella microorganism carries a deletion in at least one gene selected from the group consisting of aroA, aroB, aroC and aroD.

22. A fertilized egg according to claim 21 wherein the attenuated Salmonella microorganism carries a deletion in aroA.

23. A fertilized egg according to claim 20 wherein the attenuated Salmonella microorganism further carries a mutation in a gene encoding an enzyme of a biosynthetic pathway other than an Aro pathway.

26. A fertilized egg according to claim 13 wherein the attenuated Salmonella microorganism competitively excludes pathogenic microorganisms from a newly-hatched bird.

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L7: Entry 3 of 25 File: USPT Jan 20, 2004

DOCUMENT-IDENTIFIER: US 6680182 B1
**** See image for Certificate of Correction ****
TITLE: Expression of recombinant fusion proteins in attenuated bacteria

Brief Summary Text (18):
The promoter sequence is one having activity which is induced in response to a change in the surrounding environment, and an example of such a promoter sequence is one which has activity which is induced by anaerobic conditions. A particular example of such a promoter sequence is the nirB promoter which has been described, for example in International Patent Application PCT/GB92/00387. The nirB promoter has been isolated from E. coli, where it directs expression of an operon which includes the nitrite reductase gene nirB (Jayaraman et al, J. Mol. Biol. 196, 781-788, 1987), and nirD, nirC, cysG (Peakman et al, Eur. J. Biochem. 191, 315-323, 1990). It is regulated both by nitrite and by changes in the oxygen tension of the environment, becoming active when deprived of oxygen, (Cole, Biochem, Biophys. Acta. 162, 356-368, 1968). Response to anaerobiosis is mediated through the protein FNR, acting as a transcriptional activator, in a mechanism common to many anaerobic respiratory genes. By deletion and mutational analysis the part of the promoter which responds solely to anaerobiosis has been isolated and by comparison with other anaerobically-regulated promoters a consensus FNR-binding site has been identified (Bell et al, Nucl, Acids. Res. 17, 3865-3874, 1989; Jayaraman et al, Nucl, Acids, Res. 17, 135-145, 1989). It has also been shown that the distance between the putative FNR-binding site and the -10 homology region is critical (Bell et al, Molec. Microbiol.4, 1753-1763, 1990). It is therefore preferred to use only that part of the nirB promoter which responds solely to anaerobiosis. As used herein, references to the nirB promoter refer to the promoter itself or a part or derivative thereof which is capable of promoting expression of a coding sequence under anaerobic conditions. The preferred sequence, and which contains the nirB promoter is: AATTCAGGTAAATTTGATGTACATCAAATGGTACCCCTTGCTGAATCGTTAAGG TAGGCGGTAGGGCC (SEQ ID NO: 1)

L4: Entry 1 of 8 File: USPT Sep 21, 2004

DOCUMENT-IDENTIFIER: US 6794367 B1
TITLE: Solid preparations for oral administration of drugs relating to genes

CLAIMS:

12. The solid preparation for oral administration according to claim 2 wherein the excipient is selected from the group consisting of light anhydrous silicic acid, ethyl cellulose, carmellose, agar, magnesium aluminosilicate, calcium silicate, magnesium silicate, cyclodextrin, starch, synthetic aluminum silicate, synthetic hydrotalcite, titanium oxide, zinc oxide, magnesium oxide, alumina magnesium hydroxide, magnesium stearate, calcium stearate, aluminum silicate, talc, crystalline cellulose and lactose.

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L4: Entry 3 of 8 File: USPT Jan 16, 2001

DOCUMENT-IDENTIFIER: US 6174905 B1
** See image for Certificate of Correction **
TITLE: Cell differentiation inducer

CLAIMS:

29. The composition of claim 28, wherein said carrier or excipient is selected from the group consisting of lactose, glucose, starch, calcium carbonate, kaoline, crystalline cellulose, silicic acid, water, ethanol, propanol, simple syrup, glucose solution, starch solution, gelatin solution, carboxymethyl cellulose, shellac, methyl cellulose, polyvinyl pyrrolidone, dried starch, sodium alginate, powdered agar, calcium carmelose, a mixture of starch and lactose, sucrose, butter, hydrogenated oil, a mixture of a quarternary ammonium base and sodium lauryl sulfate, glycerine and starch, lactose, bentonite, colloidal silicic acid, talc, stearates, and polyethylene glycol.

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L4: Entry 4 of 8 File: USPT Sep 2, 1997

DOCUMENT-IDENTIFIER: US 5662935 A

** See image for Certificate of Correction **

TITLE: Process for preparing controlled release pharmaceutical forms and the forms thus obtained

CLAIMS:

8. A process according to claim 1, characterized in that for modifying the release rate at will, a substance able to inluence the hydrophily/lipophily of the composition and selected from the group consisting of polyethylene glycol, fatty acid and their salts, talc, gelatin, gum arabic, hydrogenated fats, agar, albumin, gluten and triglycerids is added to the excipient/active ingredient mixture.

19. Pharmaceutical form according to claim 10, characterized in that for modifying the release rate at will, a substance able to modify the hydrophily/lipophily of the composition and selected from the group consisting of polyethylene glycol, fatty acids and their salts, talc, gelatin, gum arabic, hydrogenated fats, agar, albumin, gluten and triglycerides is added to the excipient/active ingredient mixture.

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L4: Entry 5 of 8 File: USPT Aug 12, 1997

DOCUMENT-IDENTIFIER: US 5656284 A
TITLE: Oral transmucosal delivery tablet and method of making it

CLAIMS:

17. The tablet according to claim 16 wherein the excipient comprises glycerol and the colloidal gel comprises water and an organic polymer selected from the group consisting of agarose, agar, agar derivatives, carrageenans, algin, furcellaran, pectins, xanthan gum and locust bean gum.

22. A tablet for use in delivering a pharmaceutical transmucosally to a human, comprising an excipient not readily soluble in saliva and not including a separate adhesive or self-adhesive, and a pharmaceutical carried by the excipient, the tablet being sized to fit snugly between and in contact with a lip mucosa and an opposed gingiva mucosa and being constructed so as to be held therebetween without being adhered to either mucosa, the excipient comprising a colloidal gel which comprises water and an organic polymer selected from the group consisting of agarose, agar, agar derivatives, carrageenans, algin, furcellaran, pectins, xanthan gum and locust bean gum, the tablet having a structure which permits the pharmaceutical carried by the excipient to be delivered from the tablet at least bi-directionally to both the lip mucosa and the opposed gingiva mucosa when held therebetween.

26. A tablet for use in delivering a pharmaceutical transmucosally to a human, comprising an excipient not readily soluble in saliva and a pharmaceutical carried by the excipient, the tablet being sized to fit snugly between and in contact with a lip mucosa and an opposed gingiva mucosa and being constructed so as to be held therebetween without being adhered in the mouth, the excipient comprising a colloidal gel which comprises water and an organic polymer selected from the group consisting of agarose, agar, agar derivatives, carrageenans, algin, furcellaran, pectins, xanthan gum and locust bean gum, the tablet having a structure which permits the pharmaceutical carried by the excipient to be delivered from the tablet to either or both the lip mucosa and the opposed gingiva mucosa when held therebetween.

L4: Entry 6 of 8 File: USPT Jul 7, 1992

DOCUMENT-IDENTIFIER: US 5128143 A
TITLE: Sustained release excipient and tablet formulation

CLAIMS:

7. The excipient of claim 3, wherein said hydrophilic material further comprises one or more of tragacanth, acacia, karaya, alginates, agar, pectin, guar, hydroxypropyl guar, carrageenan, hydroxypropylmethyl cellulose, hydroxypropyl cellulose, methylcellulose, carboxymethyl cellulose, polyvinyl pyrollidone, and mixtures of any of the foregoing.

17425505 PMID: 15606352

New recombinant vaccines **based on the use of** prokaryotic **antigen-display systems.**

De Berardinis Piergiuseppe; Haigwood Nancy L

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A major challenge in vaccine design has been to identify antigen presentation systems that elicit strong T- and B-cell responses. In the authors' laboratory, two new delivery vehicles derived from nonpathogenic prokaryotic organisms were recently designed and investigated. Conserved antigenic determinants were inserted into the N-terminal region of the major pVIII coat protein of bacteriophage fd virions or on the surface of an icosahedral scaffold formed by the acyltransferase component (E2 protein) of the pyruvate dehydrogenase complex of Bacillus stearothermophilus. The data indicate that the antigenic determinant displayed by either fd virions or on the surface of the E2 lattice are accessible to the immune system, and are able to trigger a humoral response as well as a potent helper and cytolytic response in vitro and in vivo. These systems offer the potential for safe and inexpensive vaccines to elicit full-spectrum immune responses.

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☐ 1. Document ID: US 6806070 B1

L6: Entry 1 of 16 File: USPT Oct 19, 2004

DOCUMENT-IDENTIFIER: US 6806070 B1
TITLE: Use of bacterial extracts of the pseudomonadaceae family as cosmetic agents

CLAIMS:

26. A cosmetic composition comprising, as an active ingredient, a biomass obtained from a culture of at least one bacterium selected from the group consisting of Pseudomonas vesicularis and Pseudomonas maltophilia, in combination with an excipient that is acceptable in cosmetology, wherein said biomass is obtained by culturing said at least one in a culture medium bacterium and separating the biomass from the culture medium and optionally at least partially dehydrating, sterilizing, purifying, grinding and/or acylating the separated biomass.

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw De

☐ 2. Document ID: US 6664094 B1

L6: Entry 2 of 16 File: USPT Dec 16, 2003

DOCUMENT-IDENTIFIER: US 6664094 B1
TITLE: Neisserial vaccine free of immunologically functional PIII or class 4 proteins

CLAIMS:

9. A method for the immunization of a mammal against Neisseria infection, comprising the steps of: (a) isolating at least one antigen from a biologically pure culture of a mutant strain of Neisseria gonorrhoeae or Neisseria meningitidis incapable of producing a PIII protein or class 4 protein that (i) elicits blocking antibodies against the PIII or class 4 protein that inhibit bactericidal activity or (ii) immunologically reacts with said antibodies; (b) admixing the at least one antigen with a physiologically-acceptable excipient to form an antigenic composition; and (c) administering the antigenic composition to the mammal.

11. A method of preparing an antigenic composition capable of eliciting the production of antibodies which recognize Neisseria gonorrhoeae or Neisseria

meningitidis, the method comprising the steps of: 1) culturing a biologically pure mutant strain of Neisseria gonorrhoeae or Neisseria meningitidis incapable of producing a PIII protein or a class 4 protein that (i) elicits blocking antibodies against the PIII or class 4 protein that inhibit bactericidal activity or (ii) immunologically reacts with said blocking antibodies; 2) recovering at least one cell-surface antigen from the cultured strain; and 3) admixing an amount of the at least one recovered antigen sufficient to induce a host administered with the composition to produce antibodies which recognize Neisseria gonorrhoeae or Neisseria meningitidis with a physiologically acceptable excipient.

15. A method of producing antibodies in a host, which antibodies recognize Neisseria gonorrhoeae or Neisseria meningitidis, the method comprising the steps of: (a) isolating at least one antigen from a biologically pure culture of a mutant strain of Neisseria gonorrhoeae or Neisseria meningitidis incapable of producing a PIII protein or class 4 protein that (i) elicits blocking antibodies against the PIII protein or the class 4 protein that inhibit bactericidal activity or (ii) immunologically reacts with said blocking antibodies; (b) admixing the at least one antigen with a physiologically-acceptable excipient to form an admixture; (c) administering the admixture to the host.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 3. Document ID: US 6656466 B1

L6: Entry 3 of 16 File: USPT Dec 2, 2003

DOCUMENT-IDENTIFIER: US 6656466 B1
**** See image for Certificate of Correction ****
TITLE: Human tumor necrosis factor--immunoglobulin(TNFR1-IgG1) chimera composition

CLAIMS:

2. A therapeutic composition comprising human TNFR1-IgG.sub.1 prepared by a process comprising: (a) culturing a mammalian dp12.CHO host cell which expresses a human TNFR1-IgG.sub.1 chimera in a growth phase under such conditions and for a period of time such that maximum cell growth is achieved; (b) culturing the host cell in a production phase: (1) in the presence of sodium butyrate at a concentration of about 1 mM to about 6 mM; (2) at an osmolality of about 350-450 mOsm; and (3) at a temperature about between 30.degree. C. and 35.degree. C., and a pharmaceutically acceptable excipient.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 4. Document ID: US 6606822 B2

L6: Entry 4 of 16 File: USPT Aug 19, 2003

DOCUMENT-IDENTIFIER: US 6673538 B1
TITLE: Methods and compositions for designing vaccines

Detailed Description Paragraph Table (5):
Acronyms of the genes comprising the 30,000 bp flanks of the ribosomal RNA operons of the genomically sequenced H. influenzae strain Rd. rrnF flanks adhC alcohol dehydrogenase, class III (HI0185) aefA conserved hypothetical transmembrane protein (HI0195) amino acid carrier protein, putative (HI0183) aroB3-dehydroquinate synthase (HI0208) aroC chorismate synthase (HI0196) aroKshikimic acid kinase I (HI0207) arsenate reductase, putative (HI0236) bcp bacterioferritin comigratory protein (HI0254) birA biotin operon repressor/biotin acetyl coenzyme A carboxylase synthetase (HI0220) brnQ branched chain amino acid transport system II carrier protein (HI0226) esterase (HI0184) esterase/lipase, putative (HI0192) dam DNA adenine methylase (HI0209) dapA dihydrodipicolinate synthetase (HI0255) deaD ATP-dependent RNA helicase (HI0231) exbB biopolymer transport protein (HI0253) exbD biopolymer transport protein (HI0252) fldA flavodoxin (HI0191) fur ferric uptake regulation protein (HI0190) gdhA glutamate dehydrogenase (HI0189) glycosyl transferase, putative (HI0258) guaA GMP synthase (HI0222) guaB inosine-5'-monophosphate dehydrogenase (HI0221) hsdS type I restriction/modification specificity protein (HI0216) hxuB heme-hemopexin utilization protein B (HI0263) hxuA heme-hemopexin utilization protein A (HI0264) menE O-succinylbenzoate--CoA ligase (HI0194) mepA penicillin-insensitive murein endopeptidase (HI0197) msbB lipid A biosynthesis (kdo)2-(lauroyl)-lipid IVA acyltransferase (HI0199) murB UDP-N-acetylenolpyruvoylglucosamine reductase (HI0268) narQnitrate/nitrite-sensor protein (HI0267) nhaA Na+/H+ antiporter (HI0225) oligopeptide transporter, periplasmic-binding protein, putative (HI0213) pfl formate acetyltransferase (HI0180) formate transporter (HI0181) pgpB phosphatidylglycerophosphatase B (HI0211) pnp polynucleotide phosphorylase (HI0229) prlC oligopeptidase A (HI0214) queA queuosine biosynthesis protein (HI0245) rarD rarD protein, putative (HI0223) ribA GTP cyclohydrolase II (HI0212) rpL19 ribosomal protein L19 (HI0201) rpS16 ribosomal protein S16 (HI0204) secDprotein-export membrane protein (HI0240) secF protein-export membrane protein (HI0239) selD selenide, water dikinase (HI0200) seqA seqA protein (HI0193) sigma(54) sigma(54) modulation protein, putative (HI0257) ssb single-stranded DNA binding protein (HI0250) sugar kinase, putative (HI0182) tgt tRNA-guanine transglycosylase (HI0244) transcriptional regulator, putative (HI0186) tonB tonB protein (HI0251) trmDtRNA (guanine-N1)-methyltransferase (HI0202) uvrA excinuclease ABC, subunit A (HI0249) 5'-nucleotidase, putative (HI0206)

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Biotechnology in the development of new vaccines and diagnostic reagents against tuberculosis.

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Tuberculosis (TB) is a disease of global concern. About one third of the world population is infected with Mycobacterium tuberculosis. Every year, approximately 8 million people get the disease and 2 million die of TB. The currently available vaccine against TB is the attenuated strain of Mycobacterium bovis, Bacillus Calmette Guerin (BCG), which has **failed** to provide consistent protection in different parts of the world. The commonly used diagnostic reagent for TB is the purified protein derivative (PPD) of M. tuberculosis, which is nonspecific because of the presence of antigens **crossreactive** with BCG and environmental mycobacteria. Thus there is a need to identify M. tuberculosis antigens as candidates for new protective **vaccines** and **specific** diagnostic reagents against TB. By using the techniques of recombinant DNA, synthetic peptides, antigen-specific antibodies and T cells etc., several major antigens of M. tuberculosis have been identified, e.g. heat shock protein (hsp)60, hsp70, Ag85, ESAT-6 and CFP10 etc. These antigens have shown promise as new candidate vaccines and/or diagnostic reagents against TB. In addition, recent comparisons of the genome sequence of M. tuberculosis with BCG and other mycobacteria have unraveled M. tuberculosis specific regions and genes. Expression and immunological evaluation of these regions and genes can potentially identify most of the antigens of M. tuberculosis important for developing new **vaccines** and **specific** diagnostic reagents against TB. Moreover, advances in identification of proper adjuvant and delivery systems can potentially overcome the problem of poor immunogenicity/short-lived immunity associated with protein and peptide based vaccines. In conclusion, the advances in biotechnology are contributing significantly in the process of developing new protective vaccines and diagnostic reagents against TB. (120 Refs.)

Tags: Research Support, Non-U.S. Gov't
Descriptors: ***Bacteria** l Vaccines--chemical synthesis--CS; *Biotechnology--methods--MT; *Mycobacterium tuberculosis--genetics--GE; *Technology, Pharmaceutical--methods--MT; *Tuberculosis--drug therapy--DT; *Vaccines, DNA--therapeutic use--TU; Animals; **Bacterial** Vaccines --immunology--IM; **Bacterial** Vaccines--pharmacology--PD; **Bacterial** Vaccines--therapeutic use--TU; Biotechnology--trends--TD; Humans; Indicators and Reagents; Mycobacterium tuberculosis--drug effects--DE; Mycobacterium tuberculosis--immunology--IM; Reagent Kits, Diagnostic --trends--TD; Technology, Pharmaceutical--trends--TD; Tuberculosis --immunology--IM; Vaccines, DNA--pharmacology--PD
CAS Registry No.: 0 (Bacterial Vaccines); 0 (Indicators and Reagents) ; 0 (Reagent Kits, Diagnostic); 0

DOCUMENT-IDENTIFIER: US 5855880 A
** See image for Certificate of Correction **
TITLE: Avirulent microbes and uses therefor

Detailed Description Text (6):
In another embodiment of the invention, the S. typhi which are cva mutants and/or crp mutants are further mutated, preferably by a deletion, in a gene adjacent to the crp gene which governs virulence of Salmonella. Mutation in this gene, the cdt gene, diminishes the ability of the bacteria to effectively colonize deep tissues, e.g., the spleen. When a plasmid having the crp.sup.+ gene is placed in a strain with the .DELTA.(crp-cdt), it retains its avirulence and immunogenicity thus having a phenotype similar to cya and crp mutants. Mutants with the .DELTA.(crp-cdt) mutation containing a crp.sup.+ gene on a plasmid retain the normal ability to colonize the intestinal tract and GALT, but have a diminished ability to colonize deeper tissues. In the Examples, the original .DELTA.(crp-cdt) mutation as isolated in .chi.3622 also deleted the argD and cysG genes imposing requirements for arginine and cysteine for growth; this mutant allele has been named .DELTA.(crp-cysG)-10. A second mutant containing a shorter deletion was isolated that did not impose an arginine requirement; it is present in .chi.3931 and has been named .DELTA.(crp-cysG)-14. Mutations in cdt in S. typhi can be either created directly, or can be introduced via transposition from S. typhimurium strains such as those shown in the Examples. In addition, the cdt mutation can be created in other strains of Salmonella using techniques known in the art, and phenotypic selection using the characteristics described herein; alternatively, the .DELTA.cdt mutation can be transposed from the S. typhimurium described in the Examples into other strains of Salmonella using techniques of transposon mutagenesis which are known in the art.

Detailed Description Text (80):
Isolation of S. typhimurium strain with the .DELTA.crp-10 mutation. As described in Example 1, one of ten .DELTA.crp mutations isolated in .chi.3605 conferred auxotrophy for arginine (due to deletion of argD) and cysteine (due to deletion of cysG). The mutation in the S. typhimurium SL1344 strain .chi.3622 was originally referred to as .DELTA.crp-10 but is now designated .DELTA.[crp-cysG]-10 because of the auxotrophy for cysteine. A group of five BALB/c mice orally infected with 10.sup.9 .chi.3622 cells remained healthy and was totally unaffected (Table 3). Furthermore, these mice gained high-level immunity to oral challenge with 10.sup.8 parental .chi.3339 cells (Table 3).

Detailed Description Text (81):
A series of strains was constructed to independently evaluate each of the phenotypic characteristics of .chi.3622. The plasmid, pSD110, carrying the cloned crp.sup.+ gene and conferring ampicillin resistance (Schroeder and Dobrogosz, J. Bacteriol. 167:616-622 (1986)), was used to complement the .DELTA.crp mutation in the chromosome. An L broth culture of .chi.3622 was transduced with P22HTint propagated on S. typhimurium .chi.3670, which contains the plasmid pSD110. Selection was made on MacConkey agar+1% maltose+100 .mu.g ampicillin/ml. After 26 h, an ampicillin-resistant, Mal.sup.+ colony was picked and purified on MacConkey agar+1% maltose agar+100 .mu.g ampicillin/ml and designated .chi.3706. .chi.3706 was administered perorally to mice and reisolated from the spleen. The animal-passaged strain was designated .chi.3737. Two other crp mutants, .chi.3605 (crp-773::Tn10) and .chi.3623 (.DELTA.crp-11) that do not confer the Arg.sup.- or Cys.sup.- auxotrophic traits were also complemented with the pSD110 plasmid by transduction and designated .chi.3731 and .chi.3774, respectively. S. typhimurium strains independently carrying cysG and arg mutations were constructed and designated .chi.3910 (cysG::Tn10), .chi.4063 and .chi.4071 (arg::Tn10).

Detailed Description Text (84):
Virulence of the S. typhimurium .chi.3622, .chi.3731, .chi.3737, .chi.3774, .chi.3910, .chi.4063

and .chi.4071. Table 6 presents data on morbidity and mortality of mice infected perorally with the S. typhimurium strains .chi.3622, .chi.3731, .chi.3737, .chi.3774, .chi.3910, .chi.4063 and .chi.4071. Strain .chi.3737 was completely avirulent for mice that received 10.sup.4 times the LD.sub.50 dose for the wild-type .chi.3339 parent strain. Mice never appeared ill throughout the 30-day observation period. As a control for this experiment, the crp-773::Tn10 mutation in .chi.3605 was complemented by pSD110 to the wild-type Crp.sup.+ phenotype (.chi.3731) and mice were infected and died. Doses around 1.times.10.sup.5 CFU killed 4 of 5 mice p.o. inoculated with .chi.3731 and .chi.3774 (pSD110.sup.+/.DELTA.Crp-11). To test the virulence of strains with the Cys.sup.- and Arg.sup.- phenotypes independently, strains .chi.3910 (cysG::Tn10), .chi.4063 (arg::Tn10) and .chi.4071 (arg::Tn10) were p.o. administered to BALB/c mice. .chi.3910, .chi.4063 and .chi.4071 killed mice when similar or lower doses were p.o. administered. Therefore, the avirulence associated with the .DELTA.[crp-cysG]-10 mutation was not solely due to deletion of the crp gene and was not conferred by deletion of either the argD or cysG loci. Rather, another gene necessary for S. typhimurium virulence must be localized to the region of chromosome near the crp gene.

Detailed Description Text (86):
Isolation of S. typhimurium strain with the .DELTA.crp-14 mutation. Since an imprecise excision event of crp-773::Tn10 generated the deletion of genes extending from argD through cysG, another strategy was designed to locate the position of the gene conferring avirulence in the region adjacent to crp. Twenty independent deletion mutants of .chi.3910 (cysG::Tn10) were selected on fusaric acid-containing medium and screened for tetracycline-sensitivity and maltose-negative phenotype. One of twenty fusaric acid-resistant derivative of .chi.3910 had the genotype .DELTA.[crp-cysG]-14 and conferred auxotrophy for histidine and cysteine, but not arginine. This strain, designated .chi.3931, was transduced with a P22HTint lysate-grown on .chi.3670 to introduce pSD110 carrying the wild-type crp.sup.+ gene. An ampicillin-resistant, maltose-positive transductant was picked and purified on the same medium and the resulting strain was designated .chi.3955.

Detailed Description Text (91):
Based on isolation and analysis of deletion mutations for phenotypes conferred, the order of genes in the S. typhimurium chromosome is inferred to be argD crp cdt cysG.

Detailed Description Paragraph Table (1):
TABLE 1 _____ Bacterial strains Strain number Relevant genotype Derivation _____ A. E. coli CA8445 pSD110 (crp.sup.+ Ap.sup.r)/ Schroeder and Dobrogosz, J. Bacteriol. .DELTA.crp-45 .DELTA.cya-06 167:616-622 (1986). .chi.6060 F' traD36 proA.sup.+ Goldschmidt, Thoren-Gordon and proB.sup.+ lacI.sup.q Curtiss, J. Bacteriol. 172:3988-4001 .DELTA.lacZM15::Tn5/ (1990). araD139 .DELTA.(ara, leu)-7697 .DELTA.lacX74 .DELTA.phoA20 galE galK recA rpsE argE.sub.am rpoB thi B. S. typhimurium 798 wild-type prototroph Received from R. Wood, NADC, Ames, IA, as a swine isolate. #30875 wild-type prototroph Received from P. McDonough, Cornell Univ. NY as a horse isolate. DU8802 zhc-1431::Tn10 Sanderson and Roth, Microbiol. Rev. 42:485-532 (1988) PP1002 cya::Tn10 Postma, Keizer and Koolwijk, J. Bacteriol. 168:1107-1111 (1986). PP1037 crp-773::Tn10 Postma, Keizer and Koolwijk, supra. SGSC452 leu hsdLT galE Sanderson and Roth, 1988 supra. trpD2 rpsL120 metE551 metA22 hsdSA hsdSB ilv TT172 cysG::Tn10 Sanderson and Roth, 1986 supra. TT2104 zid-62::Tn10 Sanderson and Roth, supra. .chi.3000 LT2-Z prototroph Gulig and Curtiss, Infect. Immun. 55:2891-2901 (1987). .chi.3140 SR-11 wild-type Gulig and Curtiss, 1987 supra. prototroph .chi.3306 SR-11 gyrA1816 Gulig and Curtiss, 1987 supra. .chi.3385 LT-2 hsdL6 Tinge and Curtiss, J. Bacteriol. 172: galE496 trpB2 in press (1990). flaA66 his-6165 rpsL120 xyl-404 metE551 metA22 lamB.sup.+ (E. coli) .DELTA.[zja::Tn10] hsdSA29 val .chi.3339 SL1344 wild type Smith et al., Am. J. Vet. Res. 43:59-66 hisG rpsL (1984). .chi.3520 .DELTA.asdA1 zhf-4::Tn10 ATCC53681; Asd.sup.- tetracycline-resistant derivative of .chi.3000. .chi.3604 hisG rpsL P22HTint(PP1002) .fwdarw. .chi.3339 with

cya::Tn10 selection for tetracycline resistance (Mal.sup.-). .chi.3605 hisG rpsL P22HTint (PP1037) .fwdarw. .chi.3339 with crp-773::Tn10 selection for tetracycline resistance (Mal.sup.-). .chi.3615 hisG rpsL .DELTA.cya-12 Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3604. .chi.3622 hisG rpsL Fusaric acid-resistant tetracycline- .DELTA.[crp-cysG]-10 sensitive Mal.sup.- Cys.sup.- Arg.sup.- derivative of .chi.3605. .chi.3623 hisG rpsL .DELTA.crp-11 Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3605. .chi.3670 pSD110.sup.+ .chi.3385 transformed with pSD110 from galE496 trpB2 CA8445 with selection for ampicillin flaA66 his-6165 resistance, Mal.sup.+. rpsL120 xyl-404 metE551 metA22 lamB.sup.+ (E. coli) .DELTA.[zja::Tn10 hsdSA29 val .chi.3706 pSD110.sup.+ .chi.3622 transformed with pSD110 from rpsL with CA8445 selection for ampicillin .DELTA.[crp-cysG]-10 resistance, Mal.sup.+. .chi.3711 hisG rpsL .DELTA.cya-12 P22HTint(.chi.3738) .fwdarw. .chi.3615 with zid-62::Tn10 selection for tetracycline resistance, Mal.sup.-. .chi.3712 hisG rpsL .DELTA.crp-10 P22HTint(.chi.3741) .chi.3622 with zhc-1431::Tn10 selection for tetracycline resistance, Mal.sup.-, (Cys.sup.-, Arg.sup.-). .chi.3722 pSD110.sup.+ P22HTint(.chi.3711) .chi.3706 with rpsL selection for tetracycline resistance .DELTA.[crp-cysG]-10 (Mal.sup.-). .DELTA.cya-12 zid-62::Tn10 .chi.3723 pSD110.sup.+ Fusaric acid-resistant, tetracycline-hisG rpsL sensitive, ampicillin-resistant, .DELTA.(crp-cysG)-10 Mal.sup.-, Cys.sup.-, Arg.sup.- .DELTA.cya-12 derivative of .chi.3723. .DELTA.[zid-62::Tn10] .chi.3724 hisG rpsL Ampicillin-sensitive derivative of .DELTA.[crp-cysG]-10 .chi.3723; pSD110 cured by serial passage .DELTA.cya-12 in L broth at 37.degree. C. .DELTA.[zid-62::Tn10] .chi.3730 leu hsdLT galE Asd.sup.- Tc.sup.s derivative of SGSC452. trpD2 rpsL120 .DELTA.asdA1 .DELTA.(zhf-4::Tn10] metE551 metA22 hsdSA hsdSB ilv .chi.3731 pSD110.sup.+ Spleen isolate of .chi.3706 from BALB/c crp-773::Tn10 mouse. .chi.3738 zid-62::Tn10 P22HTint(TT2104) .fwdarw. .chi.3000 with selection for tetracycline resistance. .chi.3741 zhc-1431::Tn10 P22HTint(DU8802) .fwdarw. .chi.3000 with selection for tetracycline resistance. .chi.3761 UK-1 wild-type ATCC68169; Spleen isolate of #30875 prototroph from White leghorn chick. .chi.3773 hisG rpsL .DELTA.crp-11 P22HTint([.chi.3741) .fwdarw. .chi.3623 with zhc-1431::Tn10 selection for tetracycline resistance (Mal.sup.-). .chi.3774 pSD110.sup.+ .chi.3623 transformed with pSD110 from rpsL .DELTA.crp-11 CA8445 with selection for ampicillin resistance, Mal.sup.+. .chi.3777 .DELTA.[crp-cysG]-10 P22HTint(.chi.3712) 798 with selection zhc-1431::Tn10 for tetracycline resistance, Mal.sup.-, (Cys.sup.-, Arg.sup.-). .chi.3779 .DELTA.[crp-cysG]-10 P22HTint(.chi.3712) #30875 with .DELTA.[zhc-1431::Tn10] selection for tetracycline resistance, Mal.sup.-, (Cys.sup.-, Arg.sup.-). .chi.3784 .DELTA.[crp-cysG]-10 Fusaric acid-resistant, tetracycline- .DELTA.[zhc-1431::Tn10] sensitive, Mal.sup.-, Cys.sup.-, Arg.sup.- derivative . of .chi.3779. .chi.3806 .DELTA.[crp-cysG]-10 Fusaric acid-resistant, tetracycline- .DELTA. [zhc-1431::Tn10] sensitive, ampicillin-resistant, Mal.sup.-, Cys.sup.-, Arg.sup.- derivative of .chi.3777. .chi.3825 .DELTA.crp-11 P22HTint(.chi.3773) 798 with selection zhc-1431::Tn10 for tetracycline resistance, Mal.sup.-. .chi.3828 .DELTA.crp-11 P22HTint(.chi.3773) UK-1 with zhc-1431::Tn10 selection for tetracycline resistance, Mal.sup.-. .chi.3876 .DELTA.crp-11 Fusaric acid-resistant, tetracycline- .DELTA.[zhc-1431::Tn10] sensitive, Mal.sup.- derivative of .chi.3825. .chi.3901 pSD110.sup.+ P22HTint(.chi.3670) .chi.3806 with .DELTA.[crp-cysG]-10 selection for ampicillin resistance, .DELTA.[zhc-1431::Tn10] Mal.sup.+, (Cys.sup.-, Arg.sup.-). .chi.3902 pSD110.sup.+ P22HTint(.chi.3711) .chi.3901 with .DELTA.[crp-cysG]-10 selection for tetracycline resistance, .DELTA.[zhc-1431::Tn10] Mal.sup.-, (Cys.sup.-, Arg.sup.-). .DELTA.cya-12 zid-62::Tn10 .chi.3910 hisG rpsL P22HTint(TT172) .chi.3339 with cysG::Tn10 selection for tetracycline resistance, Cys.sup.-. .chi.3931 hisG rpsL Fusaric acid-resistant, tetracycline- .DELTA.[crp-cysG]-14 sensitive, Mal.sup.-, Cys.sup.-, (Arg.sup.+) derivative of .chi.3910. .chi.3936 hisG rpsL P22HTint(.chi.3711) .chi.3774 with .DELTA.crp-11 .DELTA.cya-12

Detailed Description Paragraph Table (9):

TABLE 6 _____ Virulence of S. typhimurium SL1344 .DELTA.[crp-cysG]-10, Crp.sup.+ /(crp::Tn10 and Crp.sup.+ /.DELTA.[crp-cysG]-10, arg::Tn10, cysG::Tn10 mutants in BALB/c mice 30 days after peroral inoculation Survival Strain

Relevant Inoculating live/ Mean day number genotype dose (CFU) total of death.sup.a Health.sup.b
_____ .chi.3339 wild-type 6 .times. 10.sup.4 2/5 7
scruffy .chi.3622 .DELTA.[crp-cysG]-10 6 .times. 10.sup.8 5/5 -- healthy .chi.3731 pSD110.sup.+
1 .times. 10.sup.5 1/5 9 scruffy .chi.3737 crp-773::Tn10 5 .times. 10.sup.8 5/5 - healthy
pSD110+ .DELTA.[crp-cysG]-10 .chi.3774 pSD110.sup.+ .DELTA.crp-11 3 .times. 10.sup.4 3/5 12
scruffy .chi.3910 cysG::Tn10 1 .times. 10.sup.7 0/2 12 scruffy .chi.4063 arg::Tn10 1 .times. 10.sup.9 0/2
8 scruffy .chi.4071 arg::Tn10 1 .times. 10.sup.9 0/2 9 scruffy
_____ .sup.a of animals that died .sup.b healthy -- no
noticeable signs of disease; moderate -- moderately ill; scruffy -- noticeably ill.

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ELSEVIER

Identification and characterization of a Treponema pallidum subsp. pallidum gene encoding a DNA adenine methyltransferase.

Stamm LV, Greene SR, Barnes NY, Bergen HL, Hardham JM.

Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill 27599-7400, USA. lstamm@email.unc.edu

The nucleotide sequence of a DNA adenine methyltransferase gene (dam) from Treponema pallidum has been determined. Southern blot analysis of T. pallidum chromosomal DNA indicated that this gene is present as a single copy. The dam gene encodes a 303 amino acid protein whose deduced sequence has significant homology with DNA (N6-adenine) methyltransferases. T. pallidum Dam can be assigned to group alpha DNA amino methyltransferases based on the order of nine conserved motifs that are present in the protein. Digests of T. pallidum chromosomal DNA performed with isoschizomer restriction endonucleases (Sau3AI, DpnI, and MboI) confirmed the presence of methylated adenine residues in GATC sequences (Dam+ phenotype).

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Adhesins and invasins of pathogenic bacteria: a structural view.

Niemann HH, Schubert WD, Heinz DW.

Department of Structural Biology, German Research Center for Biotechnology (GBF), Mascheroder Weg 1, 38124, Braunschweig, Germany.

Adhesion and invasion of pathogenic bacteria represent the important initial step of infection. Pathogens utilize surface-located adhesins/invasins for specific interaction with host cell receptors. The three-dimensional structures of a number of adhesins/invasins show that many are elongated molecules containing domains commonly found in eukaryotic proteins. Similar folds are employed repeatedly to target different receptors.

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WO003031599A2. 04 Oct 02. 17 Apr 03. LIVE ATTENUATED SALMONELLA STRAINS FOR PRODUCING MONOVALENT OR MULTIVALENT VACCINES. VLADOIANU, ION R, et al. C12N001/00;.

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33. US 5599537A. Salmonella vaccines contg. cells attenuated by mutation in a PhoP regulon - are highly attenuated and very safe, partic. for oral admin., mutation may opt. also be in aromatic amino acid synthesis gene.. MEKALANOS, J J, et al. A61K000/00 A61K039/02 A61K039/112 A61K039/40 C07H021/04 C12N001/20 C12N001/21 C12N001/36 C12N007/00 C12N015/00 C12N015/000 C12N015/01 C12N015/31 C12N015/32 C12N015/63 C12N015/74 C12P021/02 C12Q001/00.

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35. WO 9425598A. Eliciting an immune response to Salmonella - using attenuated Salmonella strains, vector constructs, or compsns. contg. fimbrial type proteins. CLOUTHIER, S C, et al. A61K039/112 C07K014/255 C12N001/20 C12N001:20 C12N005/12 C12N015/31 C12P021/08 C12Q001/68 C12R001:42 G01N033/56 C12N001/20 C12R001:42.

36. US 5662908A. Mutant Salmonella - has invasive phenotype imparted by hyper invasion locus gene attenuated by mutation. FALKOW, S, et al. A61K039/112 A61K039/116 C12N001/20 C12N001/21 C12N015/31 C12N015/74 C12N001/21 C12R001:42 C12N001/21 C12R001:42.

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38. EP 563311B. New anti-salmonella vaccine contg. live attenuated mutants - useful to protect against typhoid fever and related diseases, and for detecting salmonella. MEKALANOS, J J, et al. A61K039/02 A61K039/112 A61K039/40 A61P031/04 C07K014/255 C12N001/20 C12N001/21 C12N007/00 C12N015/00 C12N015/09 C12N015/63 C12N015/74 C12Q001/00 C12Q001/10 C12Q001/68 C12N001/21 C12R001:01 C12N001/21.

39. EP 555366B. Attenuation of virulent Gram-negative bacteria - by passage through phagocytes, useful as vaccines against e.g. Salmonella, in animals and humans. KRAMER, T T. A01N063/00 A61K037/00 A61K039/02 A61K039/112 A61K039/12 C12N001/00 C12N001/20 C12N001/36 C12N001/20 C12R001:42 C12N001/20 C12R001:42.

40. DD 259567A. Testing Salmonella strains for attenuation in vivo - by inoculating into intestine, then measuring cell population used to assess suitability for oral vaccines. A61K039/11.

41. RO 90248A. Attenuated live vaccine against pig Salmonellosis - prepd. by growing Salmonella cholerae bacterial strain on culture medium contg. peptone, aerating and centrifuging or sedimenting. BEN, S, et al. A61K039/11 C12N001/20.

42. AU 8290721A. Live water fowl salmonellosis vaccine - consists of aq. suspension of attenuated salmonella typhimurium. GUSCHIN, V N, et al. A61K039/11 C12N001/20 C12N007/00 C12R001/42.

43. EP 88108B. Living attenuated oral vaccine against enteric disease - comprises genetic hybrid

deriv. of salmonella typhi mutant and shigella antigen. BARON, L S, et al. A61K039/11 A61K039/112 C12N001/20 C12N001/21 C12N015/31 C12N015/87 C12N001/21 C12R001:42 C12N015/31 C12R001:01.

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Terms	Documents
salmonell$.ti. and attenuat$.ti.	44

Prev Page Next Page Go to Doc#

9. RU 2237089C. Recombinant attenuated strain of Salmonella enteriditis, useful as candidate for construction of live vaccine against human immunodeficiency virus. BOICHENKO, M N, et al. A61K039/112 A61K039/21 A61K039/295 C12N001/21 C12N001/21 C12R001:42.

11. RU 2216590C. Recombinant attenuated strain of bacterium salmonella typhimurium t10/pkhbc as producer of hepatitis b virus core antigen. BOICHENKO, M N, et al. A61K039/295 C12N001/21 C12N015/51 C12N001/21 C12R001:42.

KR2002065667A. Attenuated salmonella bacteria, useful as a live vaccine for prevention of a disease associated with Salmonella bacteria. JUN, J H, et al. C12N001/20.

13. A method of expressing chimeric **antibodies** in transfected or transformed cells, said **antibodies** comprising variable regions which bind to KSA, said method comprising the steps of:
a) constructing...

...or transformed cell is an AV12 cell.
19. A method for expressing recombinant and chimeric **antibody** chains in a recombinant non-lymphoid host cell, said method comprising:
(1) transforming said host...

...non-lymphoid host cell; and
(b) a DNA sequence that encodes a recombinant or chimeric **antibody** chain or chains, said DNA sequence being positioned for expression from said promoter and activating...

...host cell transformed in step (1) under conditions suitable for expression of recombinant or chimeric **immunoglobulin** chains.
20. The method of Claim 19, wherein said recombinant host cell is selected from...

20/3,KWIC/28 (Item 5 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
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00411080
METHOD FOR PRODUCING THERAPEUTIC DNA
PROCEDE DE PRODUCTION D'ADN THERAPEUTIQUE
Patent Applicant/Assignee:
RHONE-POULENC RORER S A,
CROUZET Joel,
CAMERON Beatrice,
Inventor(s):
CROUZET Joel,
CAMERON Beatrice,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9801540 A1 19980115
Application: WO 97FR1116 19970624 (PCT/WO FR9701116)
Priority Application: FR 968327 19960704
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AU BA BB BG BR CA CN CU CZ EE GE GH HU IL IS JP KP KR LC LK LR LT LV
MG MK MN MX NO NZ PL RO SG SI SK TR TT UA US UZ VN YU GH KE LS MW SD SZ
UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU
MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: French
Fulltext Word Count: 8400

Fulltext Availability:
Detailed Description

Detailed Description
... E.coli induit in vitro la prolifEration de cellules B murines et la sEcrEtion d' **immunoglobulines IgM** , alors que ce mEme ADN bactErien, traite in vitro avec une CpG mEthylase, n'induit...les gEnes suicides . 'Ihymidine kinase, cytosine dEsarninase, etc; ou encore tout ou partie d'une **immunoglobuline** naturelle ou artificielle (Fab, ScFv, etc), un ARN ligand (WO91/19813) etc. Le gEne thErapeutique...situEs A des

positions spEcifiques dans les sEquences. Par exemple, chez E. coli deux ADN **mEthyltransfErases** sont bien connues, l'ADN **mEthyltransfErase dam** , qui mEthyle les rEsidus adEnosine au sein des sEquences 5'- **GATC** -3', et l'ADN **mEthyltransfErase** dcm, qui mEthyle le second rEsidu cytidine des sEquences FCCA/TGG-3'. D'autres ADN...25 @d de solution de transfection ce qui correspond A l'apport de 0,375 @ **ig** d'ADN/1.10' cellules. AprEs une incubation de 2theures A 37'C sous 5...

20/3,KWIC/36 (Item 13 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
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00357011
CLONING AND EXPRESSION OF THERMOSTABLE MUTS GENES AND PROTEINS AND USES THEREFOR
CLONAGE ET EXPRESSION DE MUTANTS THERMOSTABLES DE GENES ET DE PROTEINES, ET LEURS UTILISATIONS
Patent Applicant/Assignee:
THE MOUNT SINAI MEDICAL CENTER OF THE CITY UNIVERSITY OF NEW YORK,
Inventor(s):
WETMUR James G,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9639525 A1 19961212
Application: WO 96US8677 19960604 (PCT/WO US9608677)
Priority Application: US 95468558 19950606
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
CA IL JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 22011

Fulltext Availability:
Detailed Description

Detailed Description
... Au, K.G. et al., J.
Biol. Chem. 267: 12142-12148 (1992)). In E. coli, **GATC** sites are methylated by the **dam methylase** . Hemimethylation at **GATC** permits differentiation of template from daughter strands. The repair of a mismatch is bidirectional with...proteins or polypeptides encoded by
hybridizing nucleic acids can be determined by
immunological methods employing **antibodies** that also bind to a naturally-occurring thermostable MutS protein. These methods can include immunoblot...to construct vectors and host strains, and to produce and
use the proteins, to produce **antibodies** , etc., can be applied to other members of the genus Aquifex or other members of...to construct vectors and host strains, and to produce and
use the proteins, to produce **antibodies** , etc., can also be applied to other hyperthermophilic bacteria and to thermophilic bacteria. Hyperthermophilic bacteria...

20/3,KWIC/37 (Item 14 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

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SYSTEM:OS  - DIALOG OneSearch
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         (c) 2005 Thomson Derwent & ISI
  File 348:EUROPEAN PATENTS 1978-2005/May W01
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         (c) 2005 Elsevier Science B.V.
  File 654:US Pat.Full. 1976-2005/May 05
         (c) Format only 2005 The Dialog Corp.
  File   5:Biosis Previews(R) 1969-2005/May W1
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  File 342:Derwent Patents Citation Indx 1978-05/200524
         (c) 2005 Thomson Derwent
  File 160:Gale Group PROMT(R) 1972-1989
         (c) 1999 The Gale Group
  File 345:Inpadoc/Fam.& Legal Stat 1968-2004/UD=200518
         (c) 2005 EPO
  File 370:Science 1996-1999/Jul W3
         (c) 1999 AAAS
*File 370: This file is closed (no updates). Use File 47 for more current
information.
  File 347:JAPIO Nov 1976-2005/Jan(Updated 050506)
         (c) 2005 JPO & JAPIO
  File  65:Inside Conferences 1993-2005/May W2
         (c) 2005 BLDSC all rts. reserv.
  File  94:JICST-EPlus 1985-2005/Mar W3
         (c)2005 Japan Science and Tech Corp(JST)
  File 203:AGRIS 1974-2005/Jan
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  File  16:Gale Group PROMT(R) 1990-2005/May 06
         (c) 2005 The Gale Group
*File  16: Alert feature enhanced for multiple files, duplicate
removal, customized scheduling. See HELP ALERT.
  File 340:CLAIMS(R)/US Patent 1950-05/May 05
         (c) 2005 IFI/CLAIMS(R)
*File 340: 2004 Reload is online as of October 6, 2004.  Pricing
changes effective October 1, 2004.  See HELP NEWS 340 for details.
  File 324:German Patents Fulltext 1967-200517
         (c) 2005 Univentio
*File 324: Search original German text plus English translation.
Images now available for 2005.  See HELP NEWS 324 for details.
  File  10:AGRICOLA 70-2005/Mar
         (c) format only 2005 The Dialog Corporation
  File 440:Current Contents Search(R) 1990-2005/May 09
         (c) 2005 Inst for Sci Info

      Set  Items  Description
      ---  -----  -----------
Cost is in DialUnits
? ds
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Terminal set to DLINK
? t s20/3,kwic/10 13 17 28 36 37 43 53 71 90 91 94 96

Set	Items	Description
S1	9942	(GATC OR METHYLTRANSFERASE? OR DAM) (100N) (SALMONELLA? OR TYPHIMURIUM? OR COLI)
S2	9133	S1 AND (DNA OR GENE OR PLASMID OR MUTANT? OR MUTAGEN? OR MUTATION? OR INHIBIT? OR BLOCK? OR ANTAGON? OR INACTIV? OR MODULAT? OR ALTER?)
S3	866	S2 AND ADMINISTER?
S4	63	S3 AND (DAM (10N) GENE)
S5	866	S3
S7	48	S4/1999:2005
S8	0	S4 NOT S5
S9	870	S1 AND ADMINISTER?
S10	15	S4 NOT S7
S11	206	S9 AND GATC
S12	201	S11 AND PLASMID?
S13	152	S12/1999:2005
S14	191	S12 NOT S7
S15	10	S13 NOT S14
S16	1929	(DAM OR GATC) (10N) (METHYLASE OR METHYLTRANSFERASE?)
S17	1192	RD (unique items)
S18	576	S17/1999:2005
S19	616	S17 NOT S18
S20	109	S19 AND (ANTIBOD? OR WESTERN? OR IMMUNE OR IMMUNOGLOBULIN? OR IG OR IGA OR IGG OR IGM OR ANTISERA OR ANTISERUM)

? t s20/3,kwic/10 13 17 28 36 37 43 53 71 90 91 94 96

20/3,KWIC/10 (Item 4 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS
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00499594
CD4 SPECIFIC RECOMBINANT ANTIBODY
CD4-SPEZIFISCHER REKOMBINANTER ANTIKORPER
ANTICORPS DE RECOMBINAISON SPECIFIQUE DU CD4
PATENT ASSIGNEE:
ORTHO PHARMACEUTICAL CORPORATION, (216162), Route 202, Raritan, NJ 08869-0602, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;NL;SE)
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ADAIR, John Robert, 23 George Road, Stokenchurch, High Wycombe, Buckinghamshire HP14 3RN, (GB)
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LEGAL REPRESENTATIVE:
Mercer, Christopher Paul et al (46611), Carpmaels & Ransford 43, Bloomsbury Square, London WC1A 2RA, (GB)
PATENT (CC, No, Kind, Date): EP 460178 A1 911211 (Basic)
EP 460178 B1 971015
WO 9109966 910711
APPLICATION (CC, No, Date): EP 91901835 901221; WO 90GB2015 901221
PRIORITY (CC, No, Date): GB 8928874 891221
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: C12P-021/08; C12N-015/13; A61K-039/395;

C07K-016/28; C12N-005/10; C12N-015/62;
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	9710W2	898
CLAIMS B	(German)	9710W2	788
CLAIMS B	(French)	9710W2	957
SPEC B	(English)	9710W2	13198
Total word count - document A			0
Total word count - document B			15841
Total word count - documents A + B			15841

CD4 SPECIFIC RECOMBINANT ANTIBODY

SPECIFICATION The present invention relates to CDR-grafted **antibody** molecules, to processes for their production using recombinant DNA technology and to their therapeutic uses...

...are listed in numerical order at the end of the description.
In the present application, " **Ig** " is used to describe natural **immunoglobulins** . Natural **immunoglobulins** have been known for many years and comprise a generally Y-shaped molecule having an...

...ends of the heavy chains associate to form the Fc portion.
The residue designations for **Ig** light and heavy chains given in the present description and claims are in accordance with...

...anywhere within the chains. The correct numbering of residues may be determined for a given **Ig** by alignment at regions of homology of the sequence of the **Ig** with a "standard" Kabat numbered sequence.
It was determined from a study of the amino...

...is some, but not complete, correspondence between these regions.
In the present application, the term " **antibody** " is used to describe Igs or any fragments thereof, light chain or heavy chain monomers or dimers, and single chain **antibodies** , such as a single chain Fvs in which the heavy and light chain variable domains...

...peptide linker, whether natural or produced by recombinant DNA technology or otherwise, provided that the **antibody** includes at least one antigen binding site. The remainder of the **antibody** need not comprise only **Ig** -derived protein sequences. For instance, a gene may be constructed in which a DNA sequence encoding part of a human **Ig** chain is fused to a DNA sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule. Thus, " **antibody** " encompasses hybrid **antibodies** (see below).
The abbreviation "MAb" is used to indicate a monoclonal **antibody** as produced by a hybridoma or derivative cell line.
The term "recombinant **antibody** " is used to describe an **antibody** produced by a process involving the use of recombinant DNA technology.
The term "chimeric **antibody** " is used to describe an **antibody** in which the variable domains as a whole are derived from an **antibody** from a first mammalian species and have been fused onto at least one constant domain from an **antibody** from a different mammalian species.
The term "hybrid **antibody** " is used to describe a protein comprising at least the antigen binding portion of an **Ig** attached by peptide

linkage to at least part of another protein. It will be appreciated...
...describe such constructs, but in the present specification such constructs are referred to as hybrid **antibodies** and the term chimeric **antibodies** is used in the sense defined above.

The term "CDR-grafted **antibody** " is used to describe an **antibody** having at least one, and preferably two or three, of its CDRs in one or both of the variable domains derived from an **antibody** from a first species, the remaining **Ig** -derived parts of the **antibody** being derived from one or more different **antibodies** . The variable domains may be made by use of recombinant DNA technology or by peptide...than detectable amounts, as one would expect to be produced by the untransformed host. The **antibody** of the present invention may be produced by a recombinant host cell in quantities useful...

...quantities such as about a kilogram or more.

In descriptions of processes for isolation of **antibodies** from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of **antibody** unless it is clearly specified otherwise. In other words, recovery of **antibody** from the "cells" may mean either from spun down whole cells, or from the cell...

...origin, they are naturally antigenic in humans and thus can give rise to an undesirable **immune** response, such as one response termed the Human Anti-Mouse **Antibody** (HAMA) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently...

...use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the **antibody** molecule.

In recent years advances in molecular biology based on production of a wide range...

...which codes for the variable domain of a light or a heavy chain of an **Ig** specific for a predetermined ligand. The ds DNA sequence is provided with initiation and termination...

...systems and to take advantage of the gene modification techniques proposed therein to construct chimeric **antibodies** or other modified forms of **antibody** .

It is believed that the proposals set out in the above Genentech application did not lead to the expression of any significant quantities of **Ig** polypeptide chains, nor to the production of **Ig** activity, nor to the secretion and assembly of the chains into the desired chimeric **antibodies** .

The recent emergence of techniques allowing the stable introduction of **Ig** gene DNA into mammalian cells (3) to (5) has opened up the possibility of using in vitro mutagenesis and DNA transfection to construct recombinant **antibodies** possessing novel properties.

However, it is known that the function of an **antibody** molecule is dependent on its three dimensional structure, which in turn is dependent on its primary amino acid sequence. Thus, changing the amino acid sequence of an **antibody** may adversely affect its activity. Moreover, a change in the DNA sequence coding for the **antibody** may affect the ability of the cell containing the DNA sequence to express, secrete or assemble the **antibody** .

It is therefore not at all clear that it will be possible to produce functional altered **antibodies** by recombinant DNA techniques. However, colleagues of the present inventors have devised a process whereby hybrid

antibodies in which both parts of the protein are functional can be secreted. This process is...

...No. PCT/GB85/00392. However, the above PCT application only shows the production of hybrid **antibodies** in which complete variable domains are coded for by the first part of the DNA sequence. It does not show hybrid **antibodies** in which the sequence of the variable domain has been altered.

EP-A-0 239...

...MAb have been grafted onto the framework regions of the variable domains of a human **Ig** by site directed mutagenesis using long oligonucleotides. The inventors allude to the possibility of altering...

...the heavy chain did not have a significantly altered binding activity over the CDR-grafted **antibody** with the serine to phenylalanine change at position 27 alone. These results indicate that, for CDR-grafted **antibodies** which recognize more complex antigens, changes to residues of the human sequence outside the CDR...

...antigen binding activity.

Techniques have also recently been described for altering an anti-TAC monoclonal **antibody** by CDR-grafting. Human framework regions were chosen to maximize homology with the anti-TAC **antibody** sequence, while several additional amino acids outside the CDRs were retained. The anti-TAC **antibody** so altered has an affinity for the p55 chain of human interleukin-2 of about one third that of murine anti-TAC (8).

PCT/US89/05857 also describes CDR-grafted **antibodies** which are specific for the p55 TAC protein of the IL-2 receptor. It is therein stated that the CDR-grafted **antibody** may require that 3 or more amino acid residues from the donor **Ig** in addition to the CDRs, usually at least one of which is immediately adjacent to a CDR in the donor **Ig** , be changed to correspond to that of the donor **antibody** in order to obtain antigen binding activity.

It is therefore readily apparent that it is not a simple matter to produce a CDR-grafted **antibody** . It is often not sufficient merely to graft the CDRs from a donor **Ig** onto the framework regions from an acceptor **Ig** . It may also be necessary to alter residues in the framework regions of the acceptor **antibody** in order to obtain binding activity. However, it is not possible to predict, on the...

...with the murine MAb OKT3 has shown that sometimes a population of patients develops neutralizing **antibodies** to OKT3. This **immune** response precludes repeat administration. To diminish the anticipated **immune** response to murine anti-CD4 MAbs, it would be desirable to produce a CDR-grafted version of OKT4A having murine CDRs and human framework and other **Ig** derived regions.

EP-A-0 365 209 (Becton Dickinson) describes the nucleotide and amino acid sequence of the mouse monoclonal **antibody** anti-Leu 3a which recognises the CD4 antigen. The application also describes chimeric and mosaic variants thereof.

However, as described above, the simple approach to constructing a CDR-grafted **antibody** does not always result in an **antibody** which effectively binds the antigen. The exact residues which comprise the CDRs are difficult to...

...so that they correspond to the murine residues at these positions, rendering the CDR-grafted **antibody** less "human" in character.

Despite the problems which are inherent in attempting to produce a specific CDR-grafted **antibody** , the present inventors have succeeded in producing a CDR-grafted **antibody** based on human framework regions and having an antigen binding site which is ...from the murine MAb OKT4A.

Therefore, according to the present invention, there is provided an **antibody** molecule capable of binding to the CD4 antigen comprising a composite heavy chain and a...

...domain of said composite heavy chain, the framework regions are predominantly derived from a human **antibody** (acceptor) and at least residues 23, 24, 26 to 35, 49 to 65 and 95...

...according to the Kabat numbering system) correspond to the equivalent residues in the mouse monoclonal **antibody** OKT4A (donor) as shown in Figure 3 of the accompanying drawings.

It is preferred that residues 6 and 48 in the composite heavy chain additionally correspond to the donor **antibody** in equivalent residue positions. If desired, residues 71, 73 and 79 can also so correspond...

...57, 58, 60, 88 and 91 may correspond to the equivalent residue in the donor **antibody** .

The heavy chain is preferably derived from the human KOL heavy chain as shown in...

...Kabat (1).

According to a second aspect of the present invention, there is provided an **antibody** molecule capable of binding to the CD4 antigen comprising a composite light chain and a...

...domain of said composite light chain, the framework regions are predominantly derived from a human **antibody** (acceptor) and at least residues 24 to 34, 49 to 56 and 89 to 97 (according to the Kabat numbering system) correspond to the equievalent residues in the mouse monoclonal **antibody** OKT4A (donor) as shown in Figure 4 of the accompanying drawings.

To further optimise affinity...

...in the composite light chain residue 89 corresponds to the equivalent residue in the donor **antibody** . It may also be desirable to select equivalent donor residues that form salt bridges.

The...

...be derived from the human EU light chain as shown in Kabat (1).

Preferably, the **antibody** of the first aspect of the present invention comprises as the complementary light chain a...

...appreciated that in order to retain as far as possible the human nature of the **antibody** , as few residue changes as possible should be made. It is envisaged that in many...

...it be necessary to change a larger number of framework residues.

Preferably, the CDR-grafted **antibody** is a complete **Ig** , for example of isotype IgG1)) or IgG4)).

If desired, one or more residues in the constant domains of the **Ig** may be altered in order to alter the effector functions of the constant domains.

Preferably, the **antibody** of the invention has an affinity for the CD4 antigen of between 105).M-1...

...the affinity is similar to that of MAb OKT4 or OKT4A.

Advantageously, the CDR-grafted **antibody** of the present invention is produced by use of recombinant DNA technology.

According to a third aspect of the present invention, there is provided a method for producing an **antibody** according to the first or second aspect of the present invention, which method comprises: providing...

...cell with the first DNA sequence; and culturing the transformed host cell so that an **antibody** according to the first or second aspect of the invention is produced.

Preferably, the method further comprises: providing a second DNA sequence, encoding a second **antibody** chain complementary to the first chain, under the control of suitable upstream and downstream elements...

...the first and second DNA sequences.

Advantageously, the second DNA sequence also encodes a composite **antibody** chain as defined above.

The first and second DNA sequences may be present on the...

...aspect of the present invention, there is provided a nucleotide sequence which encodes a composite **antibody** chain as defined above.

It is envisaged that the **antibodies** of the present invention will be of particular use in therapy, in particular in treating graft rejections or in treating helper T cell disorders.

The **antibodies** of the present invention may be produced by a variety of techniques, with expression in...

...being preferred. Most preferably, the host cell is a CHO host cell.

To design the **antibody** of the present invention, it is first necessary to ascertain the variable domain sequence of the mouse monoclonal **antibody** OKT4A. The variable domain sequences (VH)) and VL))) may be determined from neavy and light...framework region are preferably transferred as the "antigen binding sites", while the remainder of the **antibody** , such as the heavy and light chain constant domains and remaining framework regions, are based on human **antibodies** of different classes. Constant domains may be selected to have desired effector functions appropriate to the intended use of the **antibody** so constructed. For example, human **IgG** isotypes, IgG1)) and IgG3)) are effective for complement fixation and cell mediated lysis. For other purposes other isotypes, such as IgG2)) and IgG4)), or other classes, such as **IgM** and IgE, may be more suitable.

For human therapy, it is particularly desirable to use...

...Burroughs Wellcome Ltd.

In accordance with preferred embodiments of the present invention, certain CDR-grafted **antibodies** are provided which contain select alterations to the human-like framework region (in other words, outside of the CDRs of the variable domains) resultinq in a CDR-qrafted **antibody** with satisfactory binding affinity. Such binding affinity is preferably from about 105).M-1) to...

...affinity is about equal to that of murine MAb OKT4A.

In constructing the CDR-grafted **antibodies** of the present invention, the VH)) and/or VL)) gene segments may be altered by...

...amino acid residues or sequences contained in the Fc portion or other areas of the **antibody** may be altered in like manner (see, for example, PCT/US89/00297).

Exemplary techniques include...

...The nucleotide sequences of the present invention, capable of ultimately expressing the desired CDR-grafted **antibodies** , can be formed from a varietv of different polynucleotides (genomic DNA, cDNA, RNA or synthetic ...

...sequence comprises a fusion of cDNA and genomic DNA. The polynucleotide sequence may encode various **Ig** components (e.g. V, J, D, and C domains). They may be constructed by a...or electroporation may be used for other cellular hosts (33).

Once expressed, the CDR-grafted **antibodies** of the present invention can be purified according to standard procedures of the art, including...
...as more fully exemplified in the example section of this specification.

Substantially pure CDR-grafted **antibodies** of at least 90 to 95% homogeneity are preferred, and 98 to 99% or more...

...preferred for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the CDR-grafted **antibodies** may then be used diagnostically or therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings and the like (35).

The CDR-grafted **antibodies** of the present invention will typically find use in treating T-cell mediated disorders. For...

...macrophages, and on other tissues. The activation of these T cells can be blocked by **antibodies** recognizing the T cell receptor complex or the peptide-HLA complex. OKT3 recognizes the CD3...

...recognize HLA class II molecules. Therefore, one approach to immunosuppression involves the use of monoclonal **antibodies** , such as OKT4 or OKT4A that are immunosuppressive because they inhibit the interaction of the CD4 molecule with the HLA class II molecule. **Antibody** binding to CD4 can result in immunosuppression by a number of mechanisms including the inhibition...a subpopulation of T cells capable of suppressing other alloreactive or autoreactive subpopulations. Anti-CD4 **antibodies** may also act by inducing complement or **antibody** -dependent T cell lysis or by removal of the T cells from the blood stream or site of inflammation. Therefore the Fc-recptor binding characteristics of each **antibody** may be important to their function. Alternative strategies include the use of anti-CD4 **antibodies** that have been radiolabeled or coupled to toxins.

These immunosuppressive properties of these anti-CD4 **antibodies** provide a therapeutic use in the suppression of activated T lymphocytes that mediate the diseases...

...subunit of the HIV virus. Since OKT4A inhibits the binding of gp120 to CD4, this **antibody** or fragments thereof may block viral infection.

The CD4 molecule is normally involved in providing...

...binding to the HLA class II molecule. Therefore it is also possible that anti-CD4 **antibodies** can provide a co-stimulatory function in combination with other signal inducing reagents. This therapeutic strategy may be useful in the treatment of immunocompromised patients.

The CDR-grafted **antibodies** of the present invention may also be used in combination with other **antibodies** , particularly MAbs reactive with other markers on human cells responsible for the diseases. For example...
...as named by the First International Leukocyte Differentiation Workshop (36).

Generally, the present CDR-grafted **antibodies** will be utilized in

purified form together with pharmacologically appropriate carriers. Typically, these carriers include...

...antimicrobials, antioxidants, chelating agents and inert gases, can also be present (37).

The CDR-grafted **antibodies** of the present invention may be used as separately administered compositions or in conjunction with...

...can include "cocktails" of various cytotoxic or other agents in conjunction with the CDR-grafted **antibodies** of the present invention, or even combinations of CDR-grafted **antibodies** according to the present invention and CDR-grafted **antibodies** having different specificities.

The route of administration of pharmaceutical compositions according to the invention may...

...of ordinary skill in the art. For therapy, including without limitation immunotherapy, the CDR-grafted **antibodies** of the invention can be administered to any patient in accordance with standard techniques. The ...

...indications and other parameters to be taken into account by the clinician.

The CDR-grafted **antibodies** of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional **immunoglobulins** and art-known lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of **antibody** activity loss (e.g. with conventional **immunoglobulins** , **IgM** **antibodies** tend to have greater activity loss than **IgG** **antibodies**) and that use levels may have to be adjusted to compensate.

The compositions containing the present CDR-grafted **antibodies** or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In certain...

...upon the severity of the disease and the general state of the patient's own **immune** system, but generally range from 0.005 to 5.0 mg of CDR-grafted **antibody** per kilogram of body weight, with doses of 0.05 to 2.0 mg/kg/dose being more commonly used. For prophylactic applications, compositions containing the present CDR-grafted **antibody** or cocktails thereof may also be administered in similar or slightly lower dosages.

A composition containing a CDR-grafted **antibody** according to the present invention may be utilized in prophylactic and therapeutic settings to aid...

...collection of cells. Blood from the mammal may be combined extracorporeally with the CDR-grafted **antibodies** whereby the undesired cells are killed or otherwise removed from the blood for return to...

...mammal in accordance with standard techniques.

In addition to the therapeutic uses, the CDR-grafted **antibodies** will find use in diagnostic assays. The CDR-grafted **antibodies** may be labelled in accordance with techniques known to the art. The CDR-grafted **antibodies** are also suitable for other in vivo purposes. For example, the CDR-grafted **antibodies** can be used for selective cell treatment of peripheral blood cells where it is desired...negative controls;

Figure 18 depicts the results of studies on inhibition of MLR by various **antibodies** using T6 as negative control; and

Figure 19 depicts the results of studies on inhibition of proliferation by various **antibodies** .

Humanization of OKT4A

OKT4A is a murine monoclonal **antibody** which recognizes the CD4 antigen located primarily on helper T lymphocytes. CDR-grafted **antibodies** have been constructed in which the CDRs of the variable domains of both heavy and...

...the murine OKT4A sequence. The variable domain frameworks and constant domains were derived from human **antibody** sequences.

The three CDRs that lie on both heavy and light chains are composed of ...

...shown to be involved in antigen binding. Theoretically, if the CDRs of the murine OKT4A **antibody** were grafted onto human frameworks to form a CDR-grafted variable domain, and this variable domain were attached to human constant domains, the resulting CDR-grafted **antibody** would essentially be a human **antibody** with the specificity of murine OKT4A to bind the human CD4 antigen. Given the highly "human" nature of this **antibody** , it would be expected to be far less immunogenic than murine OKT4 when administered to patients.

Following testing for antigen binding of a CDR-grafted OKT4A **antibody** in which only the CDRs were grafted onto the human framework, it was shown that this did not produce a CDR-grafted **antibody** having reasonable affinity for the CD4 antigen. It was therefore decided that additional residues adjacent...

...from the human to the corresponding murine OKT4A residues in order to generate a functional **antibody** .

Isolation of the OKT4A heavy and light chain cDNA and DNA sequence analysis of the variable domain.

To design the CDR-grafted OKT4A **antibody** , it was first necessary to determine the sequence of the variable domain of the murine...

...sequencing primer from Pharmacia LKB Biotechnologies, Inc) has the sequence and binds to the murine **IgG** constant domain. Probe FR3 has the sequence and binds to the third framework region of...giving a proline residue at position 27 in the light chain. The first CDR-grafted **antibodies** produced by the present inventors were constructed on the assumption that light chain residue 27...

...can be seen from Figures 6, 8 and 12.

Design of the CDR-grafted OKT4A **Antibody**

To design the CDR-grafted OKT4A **antibody** , it was necessary to determine which residues of murine OKT4A comprise the CDRs of the light and heavy chains. Examination of **antibody** X-ray crystal structures shows the antigen binding surface to be located on a series...

...the crystal structure of murine OKT4A is not available, the structure of a similar murine **antibody** of known crystal structure was used to define the residues of the loops.

Three regions...

...those in the hypervariable regions would comprise composite CDRs to be grafted onto the human **antibody** framework. The amino acid sequences of the murine OKT4A heavy and light chains are presented in Figures 3 and 4, with the selected composite CDRs underlined.

The human **antibody** framework sequence for the heavy chain is that of the human **antibody** KOL. KOL was chosen because its X-ray crystallographic structure had been determined to a high degree of resolution. This should allow for accurate molecular modelling of the **antibody** . For the same reason, the framework sequence of the human light chain dimer, REI, was...

...portion. The IgG4 subclass was selected based on experience with the murine anti-CD3 monoclonal **antibody** , OKT3, which is used to treat renal graft rejection. OKT3 has a murine IgG2a isotype...

...variable domains contain signal sequences of the light and heavy chains of the murine monoclonal **antibody** B72.3 (41). The signal sequence directs secretion of the **antibody** from mammalian cells. A Kozak sequence (42) immediately precedes the AUG start codon to enhance...The regions of interest defined by nucleotide number are:

Expression of the CDR-grafted OKT4A **antibody**

Construction of the Heavy Chain Expression Vector
A CDR-grafted heavy chain expression vector was...

...EcoRI and BclI sites. The pEe6HCMVBgl2 DNA had been demethylated by passaging it through the **DAM** E. Coli strain GM242, which lacks the deoxyadenosine **methylase** . BclI will only restrict DNA which does not contain N6)-methylated deoxyadenosine at the enzyme...

...a light chain peptide is provided by a cotransfected light chain expression vector, a mature **antibody** is assembled by the binding together, via disulfide bonds, of two heavy and two light...

...removed in the rough ER. Two intrachain disulfide bonds are formed. Assembly of a mature **antibody** was discussed in the previous section.

Transient expression of CDR-grafted OKT4A in COS-1...
...in COS-1 cells provides a rapid and convenient system to test CDR-grafted OKT4A **antibody** expression and function. COS-1 cells constituitively express the SV40 large T antigen which supports...3-4 days. At that time supernatant from the wells is harvested and examined for **antibody** levels and ability to bind CD4 positive lymphocytes.
Antibody levels were determined by ELISA. Wells were coated with a goat anti-human Fc specific **antibody** . Various dilutions of the COS cell supernatant containing secreted **antibody** were added, incubated for one hour at room temperature in a humidity chamber and washed. A horse radish peroxidase-linked goat anti-human kappa chain **antibody** was added, incubated for one hour at room temperature and washed. Substrate for the horse...

...Cells were incubated at 4(degree)C for 1 hour with various dilutions of test **antibody** , positive control **antibody** or negative control **antibody** . The cells were washed once and incubated at 4(degree)C for 1 hour with an FITC-labeled goat anti-human **IgG** (Fc-specific, mouse absorbed). The cells were washed twice and analyzed by cytofluorography. chimeric OKT4A...

...incubated with mock-transfected COS cell supernatant, followed by the FITC-labeled goat anti-human **IgG** , provided the negative control.
To test the ability of CDR-grafted OKT4A to block murine...

...cells were incubated at 4(degree)C for 1 hour with various dilutions of test **antibody** or control **a**ntibody . A fixed saturating amount of FITC-OKT4A was added. The samples were incubated for 1...

...chimeric heavy chain expression vector were co-transfected into COS cells. The full chimeric OKT4 **antibody** (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding...

...is also expressed in the same expression vector as is used for the CDR-grafted **antibodies** . COS cells were co-transfected with the CDR-grafted heavy chain expression vector and the...

...or block the binding of murine OKT4A to these cells.

Modification of the CDR-Grafted **Antibody**

The binding and blocking data clearly demonstrated that the initially designed CDR-grafted OKT4A **antibody** was not capable of recognizing the CD4 antigen. Further modification of the **antibody** was necessary. Either the murine OKT4A CDRs needed to be further expanded or critical framework ...heavy chain

For modelling studies of the heavy chain the molecular model of the human **antibody** KOL was used. All residue changes were made by site-directed mutagenesis to change codons...

...binding and blocking assays is shown in Table 1. The most active CDR-grafted OKT4A **antibody** which contains the fewest murine residues is the combination of HCDR10 and LCDR2.

Alternative Light...view expressed herein that it is not necessary to change all 6 CDRs in an **antibody** in order to produce a functional CDR-grafted **antibody** .

Alternative Modifications of the CDR-Grafted Light and Heavy Chains

Residue changes made in later...

...heavy chains were done based upon molecular modelling of REI, KOL and a related mouse **antibody** , MOPC 603, rather than of the CDR-grafted **antibodies** themselves. Some of the alterations may be unnecessary for binding, especially at lower binding affinities...

...CD4 will be tested in the binding and blocking assays. The most desirable CDR-grafted **antibody** is the one with the fewest murine residues that is capable of recognizing CD4 with...

...Determination of Relative Binding Affinity

The relative binding affinities of CDR-grafted anti-CD4 monoclonal **antibodies** were determined by competition binding (8) using the HPB-ALL human T cell line as...

...antigen and fluorescein-conjugated murine OKT4A (F1-OKT4A) of known binding affinity as a tracer **antibody** . The binding affinity of F1-OKT4A tracer **antibody** was determined by a direct binding assay in which increasing amount of F1-OKT4A were...

...calibrated with quantative microbead stands (Flow Cytometry Standards, Research Triangle Park, NC). Florescence intensity per **antibody** molecule (F/P ratio) was determined by using microbeads which have a

predetermined number of mouse **IgG** **antibody** binding sites (Simply Cellular Beads, Flow Cytometry Standards). F/P equals the florescence intensity of...

...cell, and the ratio of bound/free was plotted against the number of moles of **antibody** bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor **antibody** were added to a sub-saturating dose of F1-OKT4A and incubated with 5 x...

...standards. The concentrations of bound and free F1-OKT4A were calculated. The affinities of competing **antibodies** were calculated from the equation (X) -(OKT4A) = (1/Kx) - (1/Ka), where Ka is the...

...of muring OKT4A, Kx is the affinity of competitor X ( ) is the concentration of competitor **antibody** at which bound/free binding is R/2, and R is the maximal bound/free binding.

Affinity Results

The relative affinity constants of the humanized **antibodies** (Fig. 17, Table 3) were determined, and LCDR2 combined with HCDR10 retained 68% of the activity of the parent. LCDR2/HCDR5 (Table 1) retained only 13% of the murine **antibody** affinity. These results are in agreement with those obtained in blocking assays (Fig. 16a&b...

...proliferation of peripheral blood mononuclear cells (PBMC). In order to show that the CDR-grafted **antibodies** of the present invention are likely to have the same biological activity as murine OKT4A...
...a 96 well tissue culture plate, followed by serial dilutions of a purified anti-CD4 **antibody** . Cells were cultured for 6 days, pulsed with 3)H thymidine for 24 hours and...

...control, irradiated responder cells were used in place of the irradiated allogeneic PBMC and no **antibody** was added. As a positive control, the experiment was carried out without the addition of **antibody** . In the experiment, the **antibodies** used were murine OKT4A, chimeric OKT4A and the F(ab')2)) fragment of murine OKT4APMBC were added to each well. Thereafter, serial dilutions of an anti-CD4 **antibody** were added. Cells were cultured for 72 hours, pulsed with 3)H-thymidine for 24...

...negative control, proliferation was measured in the absence of both the OKT3 and anti-CD4 **antibodies** . As a positive control proliferation was measured in the presence of OKT3 alone. In this experiment, the **antibodies** used were murine OKT4A, chimeric OKT4A and the F(ab')2)) fragment of murine OKT4A...

...chimeric OKT4A has equivalent biological properties to murine OKT4A. Since the CDR-grafted anti-CD4 **antibodies** have substantially the same affinity for the CD4 antigen as the chimeric OKT4A **antibody** and since the chimeric OKT4A **antibody** has the same constant domains as the CDR-grafted OKT4A **antibodies** , it can be expected that the CDR-grafted OKT4A **antibodies** will have the same biological functions as murine OKT4A and will thus be of use in therapy.

SUMMARY

A number of different CDR-grafted OKT4A **antibodies** have been generated. Essentially, DNA encoding the CDRs of the murine OKT4A heavy and light...

...been grafted onto the frameworks of the human heavy chain KOL and light chain REI **antibody** genes. These variable domains are ligated to the DNA encoding human kappa light chain and...

...heavy chain constant portion. The resulting CDR-grafted genes are expressed in COS-1 cells. **Antibody** secreted into the tissue culture media is collected, quantified by ELISA, and tested for its...

...positive cells and to block the binding of murine OKT4A.
The initially designed CDR-grafted **antibody** was unable to interact with CD4. A number of modifications were made to the light...

...Several of these heavy chains, in combination with LCDR2, competed well with the murine OKT4A **antibody** for CD4. Presently the CDR-grafted OKT4A of choice is the combination of LCDR2Q and...

...to the murine residues are being changed back to human. These more humanized CDR-grafted **antibodies** will be tested for their ability to recognize CD4.

REFERENCES

(1) Kabat et al., in...

CLAIMS 1. An **antibody** molecule capable of binding to the CD4 antigen comprising a composite heavy chain and a...

...domain of said composite heavy chain, the framework regions are predominantly derived from a human **antibody** (acceptor) and at least residues 23, 24, 26 to 35, 49 to 65 and 95...

...according to the Kabat numbering system) correspond to the equivalent residues in the mouse monoclonal **antibody** OKT4A (donor) as shown in Figure 3 of the accompanying drawings.

2. The **antibody** molecule of claim 1, wherein residues 6 and 48 in the composite heavy chain additionally correspond to the equivalent residues in the donor **antibody** .

3. The **antibody** molecule of claim 1 or claim 2, wherein residues 71, 73 and 79 in the composite heavy chain additionally correspond to the equivalent residues in the donor **antibody** .

4. The **antibody** molecule of any one of claims 1 to 3, wherein any one or any combination...

...and 91 in the composite heavy chain correspond to the equivalent residues in the donor **antibody** .

5. The **antibody** molecule of any one of claims 1 to 4, wherein the acceptor residues in the...

...human KOL heavy chain as shown in Figure 5 of the accompanying drawings.

6. The **antibody** molecule of any one of claims 1 to 5, wherein the complementary light chain is...

...domain of said composite light chain, the framework regions are predominantly derived from a first **antibody** (acceptor) and at least residues 24 to 34, 49 to 56 and 89 to 97 (according to the Kabat

numbering system) correspond to the equivalent residues in the mouse monoclonal **antibody** OKT4A (donor) as shown in Figure 4 of the accompanying drawings.

7. An **antibody** molecule capable of binding to the CD4 antigen comprising a composite light chain and a...

...domain of said composite light chain, the framework regions are predominantly derived from a human **antibody** (acceptor) and at least residues 24 to 34, 49 to 56 and 89 to 97 (according to the Kabat numbering system) correspond to the equivalent residues in the mouse monoclonal **antibody** OKT4A (donor) as shown in Figure 4 of the accompanying drawings.

8. The **antibody** molecule of claim 6 or claim 7, wherein residue 89 in the composite light chain additionally corresponds to the equivalent residue in the donor **antibody** .

9. The **antibody** molecule of any one of claims 6 to 8, wherein the acceptor residues in the...

...human REI light chain as shown in Figure 6 of the accompanying drawings.

10. The **antibody** molecule of any one of claims 1 to 9, which has an affinity for the CD4 antigen of from 105).M-1) to 1012).M-1).
11. The **antibody** molecule of claim 10, which has an affinity for the CD4 antigen of at least about 108).M-1).
12. The **antibody** molecule of claim 10 or claim 11, which has an affinity for the CD4 antigen similar to that of OKT4A.
13. The **antibody** molecule of any one of claims 1 to 12, which is a complete **Ig** .
14. The **antibody** molecule of claim 13, which is of isotype IgG4)).
15. The **antibody** molecule of claim 13 or claim 14, wherein one or more residues in the constant domains of the **Ig** has been altered in order to alter the effector functions of the constant domains.
16. The **antibody** molecule of any one of claims 1 to 15 which is produced by use of recombinant DNA technology.
17. A method for producing an **antibody** molecule according to any one of claims 1 to 16, which method comprises: providing a...

...cell with the first DNA sequence; and culturing the transformed host cell so that an **antibody** molecule according to any one of claims 1 to 18 is produced.

18. The method of claim 17, which further comprises: providing a second DNA sequence, encoding an **antibody** light or heavy chain complementary to the first chain, under the control of suitable upstream...

...sequences.

19. The method of claim 18, wherein the second DNA sequence encodes a composite **antibody** chain.
20. The method of claim 18 or claim 19, wherein the first and second...

...wherein the host cell is aCHO cell.

25. A nucleotide sequence which encodes a composite **antibody** chain as defined in any one of claims 1 to 9.
26. The **antibody** molecule of any one of claims 1 to 16, for use in therapy, in particular...

...graft rejections or in treating helper T cell disorders.

27. A pharmaceutical composition comprising the **antibody** molecule of any one of claims 1 to 16 in combination with a pharmaceutically acceptable...

...CLAIMS aufweist.

13. Antikorpermolekul nach einem der Anspruche 1 bis 12, wobei es sich um vollstandiges **Ig** handelt.
14. Antikorpermolekul nach Anspruch 13, das vom Isotyp IgG4)) ist.
15. Antikorpermolekul nach Anspruch 13 oder 14, wobei einer oder mehrere Reste in den konstanten Domanen des **Ig** verandert worden sind, um die Effektorfunktionen der konstanten Domanen zu verandern.
16. Antikorpermolekul nach einem...

...CLAIMS Molecule d'anticorps selon l'une quelconque des revendications 1 a 12 qui est une **Ig** entiere.

14. Molecule d'anticorps selon la revendication 13, qui est de l'isotype IgG4...

...ou 14, dans laquelle un ou plusieurs residus situes dans les domaines constants de l' **Ig** a ete modifie ou ont ete modifies dans le but de modifier les fonctions effectrices...

20/3,KWIC/13 (Item 7 from file: 348)

DIALOG(R)File 348:EUROPEAN PATENTS
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00480816

Vectors and compounds for expression of glycosylation mutants of human protein C

Vektoren und Zusammensetzungen zur Expression von Glykosilationsmutanten des menschlichen Proteins-C

Vecteurs et composes pour l'expression de mutantes de glycosylation de proteine C humaine

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	CLAIMS B	(German)	EPAB97	2007
	CLAIMS B	(French)	EPAB97	2121
	SPEC A	(English)	EPABF1	10506
	SPEC B	(English)	EPAB97	10373
Total word count - document A				12383
Total word count - document B				16382
Total word count - documents A + B				28765

...SPECIFICATION digestion of the plasmid with restriction enzyme BclI following isolation of plasmid DNA from a **dam** (sup -) **methylase** strain of E. coli, such as GM48 (NRRL B-15725). The novel zymogen genes can... used in the assays of Table IV were quantitated by an ELISA assay using monoclonal **antibodies** which may not have reacted with the mutants or derivatives to the same extent as the wild type molecule from which the **antibodies** were raised. Consequently, further purification and quantitation based upon protein content led to the data...min). Therefore, plasmid pGTC was prepared from E. coli host cells that lack an adenine **methylase** , such as that encoded by the **dam** gene, the product of which methylates the adenine residue in the sequence 5(min)-GATC-3(min). E. coli K12 GM48 (NRRL B-15725) lacks a functional **dam** **methylase** and so is a suitable host to use for the purpose of preparing plasmid pGTC...times in PBS. A 10 (mu)g/ml biotinylated goat anti-human protein C polyclonal **antibody** in 2.5% bovine serum albumin is added to the filter (in sufficient quantities to...

...37(degree)C for 1 hour.

Purification of protein C, for subsequent use to prepare **antibody** against protein C, can be accomplished as described by Kisiel, 1979, J. Clin. Invest. 64:761. Polyclonal **antibody** can be prepared by the procedure disclosed in Structural Concepts in Immunology and Immunochemistry by E.A. Kabat, published in 1968 by Holt, Rhinehart, and Winston. Monoclonal **antibody** , which is also suitable for use in the assay, can be prepared as disclosed in...variety of assay procedures can be successfully employed in the method. For instance, a double- **antibody** reaction can be employed in which the biotinylated goat anti protein C **antibody** is replaced with a goat anti-protein C **antibody** (**IgG**) and a biotinylated anti-goat **IgG** **antibody** .

The zymogen mutants may be purified from the cell cultures. The supernatant is removed from...

...SPECIFICATION digestion of the plasmid with restriction enzyme BclI following isolation of plasmid DNA from a **dam** -) **methylase** strain of E. coli, such as GM48 (NRRL B-15725). The novel zymogen genes can...used in the assays of Table IV were quantitated by an ELISA assay using monoclonal **antibodies** which may not have reacted with the mutants or derivatives to the same extent as the wild type molecule from which the **antibodies** were raised. Consequently, further purification and quantitation based upon protein content led to the data...3'. Therefore, plasmid pGTC was prepared from E. coli host cells that lack an adenine **methylase** , such as that encoded by the **dam** gene, the product of which methylates the adenine residue in the sequence 5'-GATC-3'. E. coli K12 GM48 (NRRL B-15725) lacks a functional **dam** **methylase** and so is a suitable host to use for the purpose of preparing plasmid pGTC...times in PBS. A 10 (mu)g/ml biotinylated goat anti-human protein C polyclonal **antibody** in 2.5% bovine serum albumin is added to the filter (in sufficient quantities to...

...37(degree)C for 1 hour.

Purification of protein C, for subsequent use to prepare **antibody** against protein C, can be accomplished as described by Kisiel, 1979, J. Clin. Invest. 64:761. Polyclonal **antibody** can be prepared by the procedure disclosed in Structural Concepts in Immunology and Immunochemistry by E.A. Kabat, published in 1968 by Holt, Rhinehart, and Winston. Monoclonal **antibody** , which is also suitable for use in the assay, can be prepared as disclosed in...variety of assay procedures can be successfully employed in the method. For instance, a double- **antibody** reaction can be employed in which the biotinylated goat anti protein C **antibody** is replaced with a goat anti-protein C **antibody** (**IgG**) and a biotinylated anti-goat **IgG** **antibody** .

The zymogen mutants may be purified from the cell cultures. The supernatant is removed from...

20/3,KWIC/17 (Item 11 from file: 348)

DIALOG(R)File 348:EUROPEAN PATENTS
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00364091

Novel recombinant and chimeric antibodies **directed against a human adenocarcinoma antigen.**

Rekombinante und chimare Antikorper gegen ein menschliches Adenocarcinoma-Antigen.

Anticorps chimeriques et recombinants diriges contre un antigene du type adenocarcinome humain.

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Novel recombinant and chimeric antibodies **directed against a human adenocarcinoma antigen.**

...ABSTRACT A2

The present invention comprises novel recombinant DNA compounds which encode monoclonal **antibody** KS1/4 and chimeric derivatives of monoclonal **antibody** KS1/4. Eukaryotic expression vectors have been constructed that comprise novel KS1/4-encoding DNA...

...SPECIFICATION A2

NOVEL RECOMBINANT AND CHIMERIC **ANTIBODIES** DIRECTED AGAINST A HUMAN ADENOCARCINOMA ANTIGEN

The present invention provides novel DNA compounds and recombinant DNA cloning vectors that encode monoclonal **antibody** KS1/4, as well as mouse/human chimeric **antibodies** derived from KS1/4. The vectors allow expression of the novel DNA compounds in non...

...these novel cloning vectors. The transformed host cells express the recombinant or chimeric KS1/4 **antibodies** , or derivatives thereof. Many of the present DNA compounds can be used to produce KS1...

...nature or in the laboratory, and the present invention also comprises these unique molecules.

Monoclonal **antibody** KS1/4 is a murine **antibody** which specifically binds to the ==40,000 dalton cell surface antigen found in high density on adenocarcinoma cells and found also on normal epithelial cells. This **antibody** has been shown to be effective for the in vitro detection of disease, as well...

...Press; 1987, 205-215; and Bumol, in Reisfeld, R.A. and Sell, S. eds. Monoclonal **Antibodies** and Cancer Therapy. New York: Alan R. Liss, Inc; 1985, 257-259.

One problem with the use of murine **antibodies** in human subjects arises when the cancer patient's **immune** system creates **antibodies** against the murine **immunoglobulins** . This **immune** response does not occur in all patients, but when it does, it results in a gradual decline in the efficacy of treatment during multiple dose regimens. The patient's **immune** response can cause a rapid clearance of the murine **antibody** from the patient's bloodstream. Such a response could also lead to more severe reactions like anaphylaxis or serum sickness. This immunogenicity precludes multiple dose administration of the **antibody** and therefore decreases the clinical value of the treatment.

Human monoclonal **antibodies** are difficult to prepare, therefore chimeric **antibodies** are constructed to avoid immunological problems. Chimeric **antibodies** comprise an antigen specific or variable region derived from one species joined with the constant...

...a different species. See, Oi and Morrison, BioTechniques 4:214-221 (1986). Inasmuch as the **immune** response is often directed against the constant region, the replacement of a murine constant region with a human constant region will greatly diminish a patient's immunological reaction. Accordingly, chimeric **antibodies** are highly desirable for the treatment of disease.

The general concept of chimeric **antibodies** has been described, yet the development of novel chimeric **antibodies** having certain specificities is still needed. The present invention discloses recombinant DNA and amino acid sequences which comprise the entire KS1/4

monoclonal **antibody** molecule. These sequences have been manipulated to express chimeric **antibodies** which have the same tissue specificity as KS1/4, but which comprise constant regions derived...

...The invention therefore will allow a therapeutic regimen with the same tissue specificity of monoclonal **antibody** KS1/4 but with greatly reduced immunological side effects.

The present invention further comprises the...

...non-lymphoid cell lines, thereby circumventing the problems often arising from dual secretion of heterologous **antibodies** in lymphoid cells.

For purposes of the present invention, as disclosed and claimed herein, the...

...an arginine residue.

Asn - an asparagine residue.

Asp - an aspartic acid residue.

C - deoxycytosine.

Chimeric **antibody** - an **antibody** comprising a variable region from one species, typically mouse, joined to a constant region from...

...dalton cell surface glyco-protein antigen of UCLA-P3 cells that is recognized by monoclonal **antibody** KS1/4.

KS1/4 - a murine monoclonal **antibody** derived from a hybridoma cell line, said **antibody** recognizing the ==40,000 dalton glycoprotein antigen found on the cell surface of P3-UCLA...

...of the adenovirus late promoter.

Lys - a lysine residue.

Met - a methionine residue.

MoAB - monoclonal **antibody** .

Nascent protein - the polypeptide produced upon translation of a mRNA transcript, prior to any post...recombinant DNA cloning vector into which a promoter has been incorporated.

Recombinant KS1/4 - monoclonal **antibody** KS1/4 molecules expressed in cells transformed by a vector which drives expression of KS1...

...CH2A5IG4.

The present invention is a recombinant DNA compound which comprises DNA encoding a monoclonal **antibody** light chain wherein the light chain is the light chain of monoclonal **antibody** KS1/4 and has an amino acid residue sequence substantially the same as: (see image...

...Furthermore, the present invention is a recombinant DNA compound which comprises DNA encoding a monoclonal **antibody** heavy chain wherein the heavy chain is the heavy chain of monoclonal **antibody** KS1/4 and has an amino acid residue sequence substantially the same as: (see image...

...document) (see image in original document)

The compounds of the present invention represent recombinant monoclonal **antibody** KS1/4 and the heretofore unknown amino acid and nucleotide sequences of KS1/4. Due...

...The present invention further comprises a recombinant DNA compound which comprises DNA encoding a chimeric **antibody** light chain comprising an antigen-specific variable region derived from a first mammalian species and...

...Furthermore, the invention also comprises a recombinant DNA compound which comprises DNA encoding a chimeric **antibody** heavy chain variable region derived from a first mammalian species and a constant region derived...

...from cDNA clones prepared from the mRNA from the hybridoma cell line which makes monoclonal **antibody** KS1/4. Plasmid pGKC2310 comprises the entire coding sequence of the light chain of monoclonal **antibody** KS1/4, the coding sequence of the signal peptide associated with the light chain, and entire coding sequence of the heavy chain of monoclonal **antibody** KS1/4, the coding sequence of the signal peptide associated with the heavy chain, and...

...6 comprises the cDNA coding sequence of the natural light chain variable region of monoclonal **antibody** KS1/4, the cDNA coding sequence of the signal peptide associated with the light chain, and a genomic DNA sequence which encodes the light chain constant region of a human **immunoglobulin** . Plasmid CHKC2-6 can be conventionally isolated from E. coli K12 DH5/CHKC2-6, also...

...18 comprises the cDNA coding sequence of a derivative light chain variable region of monoclonal **antibody** KS1/4, the cDNA coding sequence of the signal peptide associated with the light chain, and a genomic DNA sequence which encodes the light chain constant region of a human **immunoglobulin** . The variation in this sequence comprises the alteration of the codon at the 3(min...

...Plasmid CH2A5 comprises the cDNA coding sequence of the heavy chain variable region of monoclonal **antibody** KS1/4, the cDNA coding sequence of the signal peptide associated with said heavy chain, and a genomic DNA sequence which encodes the heavy chain constant region of human **immunoglobulin** IgG1. Plasmid CH2A5 can be conventionally isolated from E. coli K12 MM294/CH2A5, also deposited...

...Plasmid CH2A5IG2 comprises the cDNA coding sequence of the heavy chain variable region of monoclonal **antibody** KS1/4, the cDNA coding sequence of the signal peptide associated with the heavy chain and a genomic DNA sequence which encodes the heavy chain constant region of human **immunoglobulin** IgG2. Plasmid CH2A5IG2 can be conventionally isolated from E. coli K12 DH5/CH2A5IG2, also deposited...

...Plasmid CH2A5IG3 comprises the cDNA coding sequence of the heavy chain variable region of monoclonal **antibody** KS1/4, the cDNA coding sequence of the signal peptide associated with the heavy chain and a genomic DNA sequence which encodes the heavy chain constant region of human **immunoglobulin** IgG3. Plasmid CH2A5IG3 can be conventionally isolated from E. coli K12 DH5/CH2A5IG3, also deposited...

...Plasmid CH2A5IG4 comprises the cDNA coding sequence of the heavy chain variable region of monoclonal **antibody** KS1/4, the cDNA coding sequence of the signal peptide associated with the heavy chain and a genomic DNA sequence which encodes the heavy chain constant region of human **immunoglobulin** IgG4. Plasmid CH2A5IG4 can be conventionally isolated from E. coli K12 DH5/CH2A5IG4, also deposited...

...are especially preferred for the construction of vectors for transformation and expression of the various **antibody** chains in

mammalian and other eukaryotic cells. Many mammalian host cells possess the necessary cellular...

...and proper processing of the signal peptides present on the amino-terminus of the various **antibody** chains embodied in the present invention. Some mammalian host cells also provide the post-translational modifications, such as glycosylation, that are observed in **antibody** molecules. ...pLPChd. The DNA encoding the full length cDNA which encodes the light chain of monoclonal **antibody** KS1/4 is ligated into plasmid phd to form expression vector pL-KSL. The ==1100...

...linkers. This fragment comprises the full length cDNA which encodes the heavy chain of monoclonal **antibody** KS1/4. This fragment is next ligated into BclI-digested plasmid phd to form expression...use of the particular eukaryotic promoters exemplified herein. Other promoters, such as homologous or heterologous **immunoglobulin** promoters, the SV40 late promoter or promoters from eukaryotic genes, such as for example, the...

...be readily isolated and modified for use on recombinant DNA expression vectors designed to produce **antibodies** in eukaryotic host cells. Eukaryotic promoters can also be used in tandem to drive expression of such **antibodies** . Furthermore, a large number of retroviruses are known that infect a wide range of eukaryotic...

...efficient to prepare plasmid DNA in E. coli than in other host cells. Expression of **antibodies** occurs in host cells in which the particular promoter associated with the **antibody** 's structural gene functions. Skilled artisans will understand that a variety of eukaryotic host cells can be used to express the various **antibodies** using the BK enhancer-adenovirus late promoter, so long as the host cell expresses an ...

...described in detail in Example 27.

When transformed with an expression vector that encodes an **immunoglobulin** heavy chain, AV12 cells secrete the processed heavy chain into the supernatant. However, AV12 cells...

...expression vector encoding a heavy chain. A procedure for isolating clones which express the various **immunoglobulin** chains of the present invention is presented in Example 28. Functional KS1/4 and KS1...

...and matching the different light and heavy chains of the present invention.

The expression of **immunoglobulin** light and heavy chain molecules in a non-lymphoid system con stitutes a marked advantage over lymphoid systems. Traditionally, monoclonal **antibodies** have been isolated and purified from lymphoid systems such as myeloma or hybridoma cells. Such cells often express substantial quantities of heterogenic **antibodies** . This phenomena arises from the fact that lymphoid cells naturally secrete **antibodies** . When transformed with DNA encoding a separate **antibody** , or fused with other cells which produce a distinct **antibody** , such lymphoid cells sometimes become dual secreters. Dual secreting cells lines then produce many **antibodies** with hybrid molecular structures, a large population of which are not desired. These unwanted hybrids...

...the present invention traverses this problem in that non-lymphoid cells do not naturally secrete **antibody** molecules. Therefore, the only **antibody** which is secreted into the supernatant is the homogeneous product desired.

Many modifications and variations...

...comprising a promoter that functions in the host cell and drives transcription of the such **immunoglobulin** structural genes, and if the host cell possesses the cellular machinery with which to process the signal peptides, mature **antibodies** or **antibody** chains are secreted by such cells. Under other expression conditions, such as when only **immunoglobulin** light chains are expressed by the host cell, the light chains must be isolated from the host cell.

As stated above, the vectors, methods, transformants and **antibodies** of the present invention will have a profound effect upon the battle against cancer. Monoclonal **antibody** KS1/4 has been shown to be an effective agent for the diagnosis, prognosis and treatment of adenocarcinoma by Bumol in Reisfeld, R.A. and Sell, S. eds. Monoclonal **Antibodies** and Cancer Therapy. New York: Alan R. Liss, Inc., 1985, 257-259. Spearman et al...

...the teaching of which is herein incorporated by reference, disclosed the use of a monoclonal **antibody** -vinca alkaloid conjugate in the localization and treatment of tumors. This KS1/4-DAVLB(4...

...reactivity as KS1/4 molecules derived from hybridoma cells.

The problem with using a murine **antibody** , however, is that said **antibodies** often illicit an immunological response in human subjects. This has occurred in some patients receiving treatment with KS1/4. This problem can be circumvented by using the chimeric **antibodies** of the present invention. By replacing the constant regions of KS1/4 with constant regions of human origin, the patient's **immune** system will recognize the chimeric **antibody** as "self", and therefore create fewer anti-KS1/4 **antibodies** . Furthermore, the use of a human constant region will assist in the activation of complement...

...can be used to create novel, high or low affinity derivatives. Various portions of the **antibody** may be deleted or mutated to create new **antibodies** , or portions of one chain may be replaced with a piece of another chain. X-Ray crystallographic studies will demonstrate which amino acid residues of the **antibody** appear in close proximity to amino acid residues of the antigen to which KS1/4...

...can be modified to provide negative residues near positive residues on the antigen. Such "engineered" **antibodies** will then display modified affinity to the cell surface antigen in cancer patients.

The following...in the construction of plasmid phd, from E. coli host cells that lack an adenine **methylase** , such as that encoded by the **dam** gene, the product of which methylates the adenine residue in the sequence 5(min)-GATC-3(min). E. coli K12 GM48 (NRRL B-15725) lacks a functional **dam** **methylase** and so is a suitable host to use for the purpose of preparing plasmid pLPChd1...pGKC2310 contains the entire, full-length cDNA which encodes the light chain of murine monoclonal **antibody** KS1/4. E. coli K12 MM294/pGKC2310 can be obtained from the Northern Regional Research...

...pG2A52 contains the entire, full-length cDNA which encodes the heavy chain of murine monoclonal **antibody** KS1/4. E. coli K12 MM294/pG2A52 can be obtained from the Northern Research Laboratory...

...18 contains a cDNA fragment which encodes the light chain variable region of murine monoclonal **antibody** KS1/4 joined to a genomic DNA fragment which encodes a human light chain constant...

...18 contains a single amino acid alteration from the naturally-occurring variable region of monoclonal **antibody** KS1/4. The carboxy-terminal amino acid in the wild-type variable region is an...pCHKC2-6 contains a cDNA fragment which encodes the light chain variable region of monoclonal **antibody** KS1/4 joined to a genomic DNA fragment which encodes a human light chain constant...

...pCH2A5 contains a cDNA fragment which encodes the heavy chain variable region of murine monoclonal **antibody** KS1/4 joined to a genomic DNA fragment which encodes a human heavy chain constant region of **immunoglobulin** IgG1. E. coli K12 MM294/pCH2A5 can be obtained from the Northern Regional Research Laboratories...

...pCH2A5IG2 contains a cDNA fragment which encodes the heavy chain variable region of murine monoclonal **antibody** KS1/4 joined to a genomic DNA fragment which encodes a human heavy chain constant region of **immunoglobulin** IgG2. E. coli K12 DH5/pCH2A5IG2 can be obtained from the Northern Regional Research Laboratories...

...pCH2A5IG3 contains a cDNA fragment which encodes the heavy chain variable region of murine monoclonal **antibody** KS1/4 joined to a genomic DNA fragment which encodes a human heavy chain constant region of **immunoglobulin** IgG1. E. coli K12 DH5/pCH2A5IG3 can be obtained from the Northern Regional Research Laboratories...pCH2A5IG4 contains a cDNA fragment which encodes the heavy chain variable region of murine monoclonal **antibody** KS1/4 joined to a genomic DNA fragment which encodes a human heavy chain constant region of **immunoglobulin** IgG4. E. coli K12 DH5/pCH2A5IG4 can be obtained from the Northern Regional Research Laboratories...and selection of AV12 cells. To assay for the production of a fully assembled, secreted **immunoglobulin** , one therefore should assay for the presence of a secreted heavy chain.

Example 28

Assay for **Immunoglobulin** Production

The methotrexate-resistant transformants obtained in Example 27 are grown on 100 mm(sup...

...goat anti-human heavy chain (Vector Laboratories, Inc., 30 Ingold Rd., Burlingame, CA 94010) polyclonal **antibody** in 2.5% bovine serum albumin is added to the filter (in sufficient quantities to cover the filter), which is then incubated at 37(degree)C for 1 hour.

Polyclonal **antibody** can be prepared by the procedure disclosed in Structural Concepts in Immunology and Immunochemistry by E.A. Kabat, published in 1968 by Holt, Rhinehart, and Winston. Monoclonal **antibody** , which is also suitable for use in the assay, can be prepared as disclosed in...filters, which are incubated at room temperature until the color develops. Colonies secreting the most **antibody** of the invention will be indicated on the filters not only by earliest appearance of...

...which colonies are associated with which spots on the filter. The colonies secreting the most **antibody** are then selected and used for production of the **antibody** .

Those skilled in the art will recognize that the above assay is merely illustrative of...

...variety of assay procedures can be successfully employed in the method. For instance, a double- **antibody** reaction can be employed in which the biotinylated goat anti human heavy chain **antibody** is replaced with a

goat anti-human heavy chain (**IgG**) and a biotinylated anti-goat **IgG antibody** .

...CLAIMS A2

1. A recombinant DNA compound that comprises DNA encoding an **antibody** light chain with the amino acid residue sequence consisting essentially of: (see image in original...

...plasmid pGKC2310 (NRRL B-18356).

6. A recombinant DNA compound that comprises DNA encoding an **antibody** heavy chain with the amino acid residue sequence consisting essentially of: (see image in original...

...to bind to KSA.

13. A recombinant DNA compound that comprises DNA encoding a chimeric **antibody** light chain comprising an antigen-specific variable region derived from a first mammalian species and...

...described in Example 21.

19. A recombinant DNA compound that comprises DNA encoding a chimeric **antibody** light chain comprising an antigen-specific variable region derived from a first mammalian species and...

...6 (NRRL B-18358).

23. A recombinant DNA compound that comprises DNA encoding a chimeric **antibody** heavy chain comprising an antigen specific variable region derived from a first mammalian species and...

...pH4-HD as described in Example 26.

29. A method for expressing recombinant and chimeric **antibody** chains in a recombinant non-lymphoid host cell, said method comprising:
(1) transforming said host...

...non-lymphoid host cell; and
(b) a DNA sequence that encodes a recombinant or chimeric **antibody** chain or chains, said DNA sequence being positioned for expression from said promoter and activating...

...host cell transformed in step (1) under conditions suitable for expression of recombinant or chimeric **immunoglobulin** chains.

30. The method of Claim 29, wherein said recombinant host cell is selected from...plasmid pH2-HD, plasmid pH3-HD or plasmid pH4-Hd.

33. A recombinantly produced monoclonal **antibody** comprising a light chain with the amino acid sequence consisting essentially of: (see image in original document)

34. A recombinantly produced monoclonal **antibody** comprising a heavy chain with the amino acid sequence consisting essentially of: (see image in original document) (see image in original document)

35. A recombinantly produced monoclonal **antibody** comprising a light chain with the amino acid sequence consisting essentially of: (see image in...

...essentially of: (see image in original document) (see image in original document)

36. A monoclonal **antibody** light chain signal peptide with an amino acid sequence consisting essentially of: (see image in original document)

37. A monoclonal **antibody** heavy chain signal peptide with an amino acid sequence consisting essentially of: (see image in original

document)
38. A chimeric monoclonal **antibody** comprising an antigen-specific variable region derived from a first mammalian species and a constant ...

...amino acid sequence consisting essentially of: (see image in original document)
39. A chimeric monoclonal **antibody** comprising an antigen-specific variable region derived from a first mammalian species and a constant ...

...amino acid sequence consisting essentially of: (see image in original document)
40. A chimeric monoclonal **antibody** comprising an antigen-specific variable region derived from a first mammalian species and a constant ...

...amino acid sequence consisting essentially of: (see image in original document)
41. A chimeric monoclonal **antibody** comprising an antigen-specific variable region derived from a first mammalian species and a constant ...

...the following Contracting State: GR
1. A recombinant DNA compound that comprises DNA encoding an **antibody** light chain with the amino acid residue sequence consisting essentially of: (see image in original...

...plasmid pGKC2310 (NRRL B-18356).
6. A recombinant DNA compound that comprises DNA encoding an **antibody** heavy chain with the amino acid residue sequence consisting essentially of: (see image in original...

...to bind to KSA.
13. A recombinant DNA compound that comprises DNA encoding a chimeric **antibody** light chain comprising an antigen-specific variable region derived from a first mammalian species and...

...described in Example 21.
19. A recombinant DNA compound that comprises DNA encoding a chimeric **antibody** light chain comprising an antigen-specific variable region derived from a first mammalian species and **antibody** heavy chain comprising an antigen specific variable region derived from a first mammalian species and...

...pH4-HD as described in Example 26.
29. A method for expressing recombinant and chimeric **antibody** chains in a recombinant non-lymphoid host cell, said method comprising:
(1) transforming said host...

...non-lymphoid host cell; and
(b) a DNA sequence that encodes a recombinant or chimeric **antibody** chain or chains, said DNA sequence being positioned for expression from said promoter and activating...

...host cell transformed in step (1) under conditions suitable for expression of recombinant or chimeric **immunoglobulin** chains.
30. The method of Claim 29, wherein said recombinant host cell is

selected from...

...plasmid pH2-HD, plasmid pH3-HD or plasmid pH4-HD.

33. A recombinantly produced monoclonal **antibody** comprising a light chain with the amino acid sequence consisting essentially of: (see image in original document)

34. A recombinantly produced monoclonal **antibody** comprising a heavy chain with the amino acid sequence consisting essentially of: (see image in...

...in original document)

Claims for the following Contracting State: ES

1. A method of expressing **antibody** chains in transformed or transfected cells, said **antibody** chains capable of binding KSA, said method comprising the steps of:
   (a) constructing a recombinant...

...conditions suitable for the expression of said light chain gene.

2. A method of expressing **antibody** chains in transformed or transfected cells, said **antibody** chains capable of binding KSA, said method comprising the steps of:
   a) constructing a recombinant...

...conditions suitable for the expression of said heavy chain gene.

3. A method of expressing **antibody** chain derivatives in transformed or transfected cells, said **antibody** chain derivatives capable of binding KSA, said method comprising the steps of:
   a) constructing a...

...suitable for the expression of said light chain derivative gene.

4. A method of expressing **antibody** chain derivatives in transformed or transfected cells, said **antibody** chain derivatives capable of binding KSA, said method comprising the steps of:
   a) constructing a...

...for the expression of said heavy chain derivative gene.

5. A method of expressing chimeric **antibody** chains in transfected or transformed cells, said **antibody** chains comprising variable regions which bind to KSA, said method comprising the steps of:
   a...

...the transfected or transformed cell is an AV12 cell.

8. A method of expressing chimeric **antibody** chains in transfected or transformed cells, said **antibody** chains comprising variable regions which bind to KSA, said method comprising the steps of:
   a...

...suitable for the expression of said light chain gene.

9. A method of expressing chimeric **antibody** chains in transfected or transformed cells, said **antibody** chains comprising variable regions which bind to KSA, said method comprising the steps of:
   a...the transfected or transformed cell is an AV12 cell.

12. A method of expressing chimeric **antibodies** in transfected or transformed cells, said **antibodies** comprising variable regions which bind to KSA, said method comprising the steps of:
   a) constructing...

...suitable for the expression of said variable region genes.

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00350763

NUCLEOTIDE SEQUENCE OF THE HAEMOPHILUS INFLUENZAE Rd GENOME, FRAGMENTS THEREOF, AND USES THEREOF

SEQUENCE NUCLEOTIDIQUE DU GENOME HAEMOPHILUS INFLUENZAE RD, DES FRAGMENTS DE CE DERNIER, AINSI QUE SES APPLICATIONS

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Fulltext Availability:
Detailed Description
Claims

Detailed Description
... colony/plaque hybridization, one skilled in the art can obtain homologs

The invention further provides **antibodies** which selectively bind one of the proteins of the present invention. Such **antibodies** include both monoclonal and polyclonal **antibodies**

SUBSTITUTE SHEET (RULE 26)
The invention further provides hybridomas which produce the above described **antibodies** . A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal **antibody**

The present invention further provides methods of identifying test samples derived from cells which express more of the **antibodies** of the present invention, or one or
more of the DFs of the present invention...

...confinement, one or more containers which comprises: (a) a first container comprising one of the **antibodies** , or one of the DFs of the present invention; and (b) one or ...one or more of the following: wash reagents, reagents capable of detecting presence of bound

antibodies or hybridized DFs

Using the isolated proteins of the present invention, the present invention further...

...protein encoded by one of the ORFs of the present invention. Specifically, such agents include **antibodies** (described above), peptides, carbohydrates, pharmaceutical agents and the like. Such methods comprise the
steps of...producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating **antibodies** against the native polypeptide. In an alternative method, the polypeptide or protein ...ligases, gyrases and
methylases, which have immediate use in the biotechnology industry

2. Generation of **Antibodies**
SUBSTITUTE SHEET (RULE 26)
As described here, the proteins of the present invention, as well...

...other proteins. The proteins of the present invention can further be used to generate an **antibody** which selectivel binds the protein. Such **antibodies** can be either monoclonal or polyclonal **antibodies** , as well fragments of these **antibodies** , and humanized forms

The invention further provides **antibodies** which selectively bind to one
of the proteins of the present invention and hybridomas which produce these **antibodies** . A hybridoma is an immortalized cell line which is capable of
secreting a specific monoclonal **antibody**

In general, techniques for preparing polyclonal and monoclonal **antibodies** as well as hybridomas capable of producing the desired **antibody** are
well known in the art (Campbell, A.M., Monoclonal **Antibody** Technology: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1984...hybridoma technique (Kozbor et al., Immunology Today 4:72 (1983); Cole et al., in Monoclonal **Antibodies**
and Cancer Therapy, Alan R. Liss, Inc. (1985), pp. 77-96)

Any animal (mouse, rabbit, etc.) which is known to produce **antibodies** can be immunized with the pseudogene polypeptide. Methods for immunization are well known in the...globulin or ss-galactosidase) or through the inclusion of an
adjuvant during immunization

For monoclonal **antibodies** , spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag 14 myeloma cells,
and allowed to become monoclonal **antibody** producing hybridoma cells Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an **antibody** with the desired characteristics. These include screening the hybridomas with an ELISA assay,
western blot analysis, or radioimmunoassay (Lutz et al., Exp. Cell Res

175:109-124 (1988))

Hybridomas secreting the desired **antibodies** are cloned and the class and subclass is determined using procedures known in the art (Campbell, A.M., Monoclonal **Antibody** Technology: Laboratory Techniques in Biochemistry and Molecular ...Elsevier Science Publishers, Amsterdam, The Netherlands (1984))

Techniques described for the production of single chain **antibodies** (U.S. Patent 4,946,778) can be adapted to produce single chain **antibodies** to
proteins of the present invention

For polyclonal **antibodies** , **antibody** containing **antisera** is isolated from
the immunized animal and is screened for the presence of **antibodies** with the
desired specificity using one of the above-described procedures

The present invention further provides the above-described **antibodies** in detectably labelled form. **Antibodies** can be detectably labelled through the use of radioisotopes, affinity labels (such as biotin, avidin ...Goding, J.W. J. Immunol. Meth. 13:215 (1976))

SUBSTITUTE SHEET (RULE 26)
The labeled **antibodies** of the present invention can be used for in vitro, in vivo, and in situ...the
Haemophilus influenzae Rd genome is expressed

The present invention further provides the above-described **antibodies** immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate...

...agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling **antibodies** to such solid supports are well known in the art (Weir, D.M. et al...

...Jacoby, W.D. et al., Meth. Enzym. 34 Academic Press, N.Y. (1974)). The immobilized **antibodies** of ...present invention, or homolog thereof, in a test sample, using one of the DFs or **antibodies** of the present invention

In detail, such methods comprise incubating a test sample with one or more of the **antibodies** or one or more of the DFs of the present invention and assaying for binding of the DFs or **antibodies** to components within the test
sample

Conditions for incubating a DF or **antibody** with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the DF or **antibody** used in the assay. One skilled in the art will recognize that any one of...

...hybridization, amplification or immunological assay
formats can readily be adapted to employ the DFs or **antibodies** of the present

invention. Examples of such assays can be found in Chard, T., An...or more containers which comprises: (a) a first container comprising one of the DFs or **antibodies** of the present invention; and (b) one or more other containers comprising one or more of the following:
wash reagents, reagents capable of detecting presence of a bound DF or **antibody**

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate...which will accept the test sample, a container which contains the
SUBSTITUTE SHEET (RULE 26)
antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the
reagents used to detect the bound **antibody** or DF

Types of detection reagents include labelled nucleic acid probes, labelled secondary **antibodies** , or in the alternative, if the primary **antibody** is labelled, the enzymatic, or **antibody** binding reagents which are capable of
reacting with the labelled **antibody** . One skilled in the art will readily
recognize that the disclosed DFs and **antibodies** of the present invention can be readily incorporated into one of the established kit formats...the organism blocking attachment or rendering the organism more prone to act the bodies nature **immune** system. Alternatively,
the agent may be comprise a protein encoded by one of the ORFs...change in the immunological character of the functional
derivative, such as affinity for a given **antibody** , is measured by a competitive
type immunoassay. Changes in immunomodulation activity are measured by the...with identified start and stop codons have also been accessioned by GSDB

Production of an **Antibody** to a Haemophilus influenzae Protein
Substantially pure protein or ...an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or
polyclonal **antibody** to the protein can then be prepared as follows:
Monoclonal **Antibody** Production by Hybridoma Fusion
Monoclonal **antibody** to epitopes of any of the peptides identified and isolated as described can ...protein over a period of a few weeks. The mouse is then sacrificed, and the **antibody** producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene... dilution placed in wells of a microtiter plate where growth of the culture is continued. **Antibodyproducing** clones are identified by detection of **antibody** in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally...
...419 (1980), and modified
methods thereof. Selected positive clones can be expanded and their monoclonal **antibody** product harvested for use. Detailed procedures for monoclonal **antibody** production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, New York. Section 21-2 (1989)

Polyclonal **Antibody** Production by Immunization
Polyclonal **antiserum** containing **antibodies** to heterogenous epitopes of
a single protein can be prepared by immunizing suitable animals with...

...expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal **antibody** production is affected by many factors related both to the antigen and the host species ...

...inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer **antisera** . Small doses (...991 (1971)

SUBSTITUTE SHEET (RULE 26)
Booster injections can be given at regular intervals, and **antiserum** harvested when **antibody** titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known...

...Chap

19 in: Handbook of Experimental Immunology, Wier, D., ed, Blackwell (1973). Plateau concentration of **antibody** is usually ...0.1 to 0.2 mg/ml of serum (about 12 SM). Affinity of the **antisera** for the antigen is
determined by preparing competitive binding curves, as described, for example, by...

...Immunology, second edition, Rose and Friedman, eds., Amer. Soc. For Microbiology, Washington,
D.C. (1980)

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of...mer peptides synthesized from the predicted Haemophilus DNA
sequence are injected into mice to generate **antibody** to the polypeptide encoded by the Haemophilus DNA
If **antibody** production is not possible, the Haemophilus DNA sequence is additionally incorporated into eukaryotic expression vectors and expressed as a chimeric with, for example, B-globin. **Antibody** to ss-globin is used to
purify the chimeric. Corresponding protease cleavage sites engineered between...H109551011537 1012736dfp protein (dfp) (Escherichia coli) 55.4 71.4 266
H10210 223259 224116DNA adenine **methylase** (**dam**) (Escherichia coli) 70.6 84.9 859
u1 H112671343755 1341116DNA gyrase, subunit A (gyrA) (Escherichia ...

Claim
... An isolated polynucleotide molecule encoding any one of the polypeptides of claim 17.

19. An **antibody** which selectively binds to any one of the polypeptides of claim 17.

20. A method...

20/3,KWIC/43 (Item 20 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
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00294539 **Image available**

METHODS OF ANALYSIS AND MANIPULATION OF DNA UTILIZING MISMATCH REPAIR SYSTEMS

PROCEDES D'ANALYSE ET DE MANIPULATION D'ADN A L'AIDE DE SYSTEMES DE REPARATION D'ERREURS D'APPARIEMENT

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CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
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Fulltext Availability:
Detailed Description
Claims

Detailed Description
... membranous nitrocellulose filter, detection of the DNA:protein complex further includes the step wherein an **antibody** specific for the base mispair recognition protein is employed, the base mispair recognition protein is...fragment of up to several hundred base pairs is known to produce distinguishable mobility differences.

Antibodies specific for a DNA mispair recognition protein can be prepared by standard immunological techniques known...

...other suitable analytical methods for detecting the DNA protein complex include immunodetection methods using an **antibody** specific for the base mispair recognition protein. For example, **antibodies** specific for the E. coli MutS protein have been prepared. Accordingly, one immunodetection method for...

...protein complexes from DNA that does not f orm such complexes by immunoprecipitation with an **antibody** specif ic for MutS protein, and detecting the DNA in the precipitate.

According to the...otherwise GATC unmodified molecules is desired, this can be accomplished by use of E. coli **Dam methylase** as is well known in the art.

Symmetrically methylated DNA prepared by use of this...at

340 C and supplemented with 2.2 ILl of a solution
containing 0.1 / **ig** (24 fmol) of hemimethylated G-T
heteroduplex DNA, 16 ng ...minus exonuclease I 2 <1
minus DNA helicase II 16 15
minus helicase II,
plus **immune** serum <1 <1
minus helicase II,
plus pre- **immune** serum 14 NT
minus Ligase/NAD* 14 NT
minus MgC12 <1 NT
minus ATP <1...

...incised
with MutH protein as described in the legend to Fig. 4.

When present, rabbit **antiserum** to helicase II or pre
immune serum (5 Ag protein) was incubated at 00 C for 20
minutes with reaction mixtures...

...the cofactor
was then added and the assay was performed as above.

Although not shown, **antiserum** inhibition was reversed by
the subsequent addition of more helicase II. with the
exception of abolished by **antiserum** to
homogeneous helicase II, suggesting a requirement for this
activity and that it might be...other methods of the invention; e.g.,
altered electrophoretic mobility, or detection by use of
antibodies .

If the above detection test indicates the
presence of sequence differences between the human tissue...

Claim
... wherein said step for
detecting,the DNA:protein complex further includes a step
wherein an **antibody** specific for said DNA base mispair
recognition protein is employed.

9 A method for detecting...

20/3,KWIC/53 (Item 30 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
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00237967
COMPOSITE ANTIBODIES **OF HUMAN SUBGROUP IV LIGHT CHAIN CAPABLE OF BINDING TO TAG-72**
COMPOSITES DE LA CHAINE LEGERE DU SOUS-GROUPE HUMAIN IV APTES A LA LIAISON AVEC TAG-72
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JOHNSON Kim S,
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Application: WO 91AU583 19911213 (PCT/WO AU9100583)
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COMPOSITE ANTIBODIES **OF HUMAN SUBGROUP IV LIGHT CHAIN CAPABLE OF BINDING TO TAG-72**

Fulltext Availability:
Detailed Description
Claims

English Abstract
This invention concerns a subset of composite Hum4 VL, VHalphaTAG **antibody** with high affinities to a high molecular weight, tumor-associated sialylated glycoprotein antigen (TAG-72) of human origin. These **antibodies** have variable regions with (1) VL segments derived from the human subgroup IV germline gene...

...to bind TAG-72. in vivo methods of treatment and diagnostic assay using these composite **antibodies** is also disclosed.

Detailed Description
COMPOSITE **ANTIBODIES** OF HUMAN SUBGROUP IV LIGHT CHAIN CAPABLE OF BINDING TO TAG-72
The present invention...

...be construed, that any of the following .Lnformation constitutes prior art against the present10 invention.

Antibodies are specific immunoglobul.in (**Ig**) polypeptides produced by the vertebrate **immune** system in reSDonse I[lo challenges by foreign proteins, 15 vlycooroteins, cells, or other antigenic...

...The binding specificity of such polypeptides to a particular antiven is highly refined.

with each **antibody** being aimost exciusively directed -,o L.he oarticuiar anti,-en which elicited i:.

SUBSTITUTE SHEET...

...Trends inBiochemSci, 6:203 (1981). Even when only a single antigen is introduced into the **immune** system for a A particular mammal, a uniform population of **antibodies** does not result, i,e,, the response is polyclonal.

The limited but inherent heterogeneity of polyclonal **antibodies** is overcome by the use of hybridoma technology to create "monoclonal" **antibodies**

in cell cultures by B cell hybridomas (see Kohler and Milstein (1975). Nature. 256:495...

...hybrid cells or "hybridomas" which are both immortal and capable of producing the genetically-coded **antibody** of the B cell,
In many applications, the use of monoclonal **antibodies** oroduced in non-human animals is severely restricted where the monoclonal **antibodies** are to be used in humans, Repeated injections in humans of a "foreign" **antibody** , such as a mouse **antibody** , may lead to harmful hypersensitivity reactions, i,e,, an anti idiotypic, or human anti-mouse **antibody** (HAMA) response:, (see Shawler etal, (1985), Journal of ImmunoloSX, 135:1530 and Sear etal.. J...

...Modifiers, 3:138-150),
Various attempts have already been made to manufacture human-derived monoclonal **antibodies** by using human hybridomas (see Olsson etal.. Proc, Natl, Acad, Sci. U.S.A., 77...

...and Roder etal. (1986),
Methods in Enzymology, 121:1r40 UnfortunatLely,
SUBSTITUTE SHEET
yields of monoclonal **antibodies** from human hybridoma cell lines are relatively low compared to mouse hybridomas. in addition, human cell lines expressing **immunoglobulins** are relatively unstable compared to mouse cell lines, and the **antibody** producing capability of these human cell lines is transient. Thus, while human **immunoglobulins** are highly desirable, human hybridoma techniques have not yet reached the stage where human monoclonal **antibodies** with required antigenic specificities can be easily obtained,
Thus, **antibodies** of nonhuman origin have been genetically engineered, or "humanized". Humanized **antibodies** reduce the HAMA response compared to that expected after injection of a human patient with a mouse **antibody** , Humanization of **antibodies** derived from nonhumans, for example, has taken two principal forms.

i,e., chimerization where non-human regions of **immunoglobulin** constant sequences are replaced by corresponding human ones (see for example, USP 498169567 to Cabilly...

...European Patent Office
Application (EPO) 0 239 400 to Winter). Some researchers have oroduced Fv **antibodies** (USP 4,642,334 to Moo-re, DNAX) and single chain Fv (SCFV) **antibodies** (see USP.4,946,778 to Ladner, Genex).

The above patent applications only show 'LI-,he oroduction of **antibodv** fraaments In which some oor+-;on of the variable domains is coded -for ty nonhuman -1 gene regions. Humanized **antibodies** to date still retain various portions of liaht and heavy chain variable

regions of nonhuman origin: the chimeric. F.v and single chain F"v **antibodies** retain the entire variable region of SUBSTITUTE SHEET nonhuman origin and CDR-grafted **antibodies** retain CDR of nonhuman origin.

Such nonhuman-derived regions are expected to elicit an immunogenic...

...J. ExD. Med. , 170:2153-2157; and Lo Buglio (1991), Sixth International Conference on Monoclonal **Antibody** Immunoconjugates for Cancer, San Diego, Ca). Thus, it is most desirable to obtain a human...

...human carcinoma tumor antigen is tumor-associated glycoprotein-72 (TAG-72), as defined by monoclonal **antibody** B72-3 (see Thor etal. C 1986) Cancer Res,, 46:3118-3124; and Johnson, etal...

...a variant of the LS180 (ATCC No. CL 187) colon adenocarcinoma line.

Numerous murine monoclonal **antibodies** have been developed which have binding specificity for TAG Exemplary ffurine monoclonal **antibodies** include the "CC" (colon cancer) monoclonal **antibodies** . which are a library of murine monoclonal **antibodies** developed using TAG-72 ourified an an immunoaffinity column with an -mmobilized anti-TAG-72 **antibody** , 372.3 (ATCC HE-8108) (see EEP 394277, to Schlom etal., National Cancer Tnstitute), Certain CC **antibodies** were der)osited with the ATECC: CC49 CATCC No. IH-B 9459); CC83 (ATCC No...

...HB 945T); CC11 (ATCC No. 9455) and CCI15 SUBSTITUTE SHEET (ATCC No. HB 9460), Various **antibodies** of the CC series have been chimerized (see, for examole, EPO 0 365 997 to Mezes etal., The Dow Chemical Company).

It is thus of great interest to develop A **antibodies** against TAG-72 containing a light and/or heavy chain variable region(s) derived from human **antibodies** . However, the prior art simply does not teach recombinant and immunologic techniques capable of routinely producing an anti-TAG-72 **antibody** in which the light chain and/or the heavy chain variable regions have ificity and ...elicit expectedly low or no HAMA response, It is known that the function of an **immunoglobulin** molecule is dependent on its three dimensional structure, which in turn is dependent on its...

...a few or even one amino acid can drastically affect the binding function of the **antibody** can drastically affect its the bidnl,ng affinity of the **antibody** , i.e., the resultant **antibodies** are generally presumed to be a non-specific

immunoglobulin (NSI), i.e., lacking in **antibody** character, (see, for examDle, USP 4,816,567 to Cabilly etal., Genentech).

Surprisingly, the present...

...meeting many of these above mentioned needs and provides a method for supplying the desired **antibodies** , For examole, in one asoect, the oresent invention provides a cell caoable of expressing a composite **antibody** having binding specif.1city for "L"AG-72, said cell being transformed with (a) a...

...ability to bind to TAG-72* in another aspect, the present invention provides a composite **antibody** or **antibody** having binding specificity for TAG-72, comprising (a) a DNA sequence encoding at least a...

...three dimensional structure having the ability to bind TAG The invention further includes the aforementioned **antibody** alone or conjugated to an imaging marker or therapeutic agent, The invention also includes a composition comprising the aforementioned **antibody** in unconjugated or conjugated form.in a pharmaceutically acceptable, non-toxic, sterile carrier.

The invention...

...localized tumors. Additionally, the invention also concerns a process for preparing and expressing a composite **antibody** . Some of these processes are as follows. A process which comprises transforming a cell with...structure having the ability to bind to TAG-72, A process for preparing a comDosite **antibody** or **antibody** which comprises culturing a cell containing a DNA sequence encoding at least a portion of...

...ability to bind to TAG-72 under sufficient conditions for the cell to express the **immunoglobulin** light chain and immuno globulin heavy chain. A process for preparing an **antibody** conjugate comprising contacting the aforementioned **antibody** or **antibody** with an imaging marker or tlnerapeutic agent, Descriotion of the Drawincs Figure 1 illustrates a basic **immunoglobulin** structure, SUBSTITUTE SHEET .Figure 2 illustrates the nucleotide sequences Of VHaTAG, CC46 VH, CC49 VH...
...VH and CC92 VH.

Figure 4 illustrates the VH nucleotide and amino acid secuences of **antibody** B17X2.

Figure 5 illustrates the mouse ge:rmline J-H
io genes from PNP9.

Figure...

...20 illustrates a competition assay for
binding to TAG-using a composite Hum4 VL, VHaTAG
antibody .

Figure 21 illustrates a general DNA
construction of a single chain, composite Hum4 VL9
VHaTAG...IUPAC IUB
(Commission on Biological Nomenclature) or the practice
in the fields concerned.

The basic **immunoglobulin** structural unit is set
forth in Figure 1. The terms "constant" and "variable"
are used...

...of light (CL) and heavy (CH)
chains confer important biological properties such as
SUBSTITUTE SHEET
antibody chain association, secretion, transplacental
complement binding, binding to Fc receptors
moo
and the like.

The **immunoglobulins** of this invention have been
developed to address the problems of the prior art, The
methods of this invention produce, and the invention is
directed to, composite **antibodies** , By "composite
antibodies " is meant **immunoglobulins** comprising variable
regions not hitherto found associated with each other in
nature. By, "composite Hum4 VL, VH **antibody** " means an
antibody or immunoreactive fragment thereof which is
characterized by having at least a portion of the...

...three dimensional
structure having IChe ability to bind to TAG
The composite Hum4 VL? VH **antibodies** of the
oresent invention assume a conformation having an
antigen binding site which binds specifically...

...sorter analysis (FACS), immunohistochemis'L-Iry and the
like). Preferably, the composite Hum4 VL, VH **antibodies**
of the present invention have an antigen binding
affinity or avidity greater than 105 M...

...SHEET
92:543-577 and Scatchard (1949), Ann, NJ. Acad, Sci.,
51:660-672*
Human **antibody** kappa chains have been
classified into four subgroups on the basis of -invariant
amino acid...Wang etal. (1973). Nature, 243:126
127e
It has been found, quite surprisingly, that an
immunoglobulin having a light chain with at least a

portion of the VL encoded by a...

...Horton etal. (1989), Gene, 77:61-68).
The CL Of the composite Hum4 VL, VH **antibodies** is not critical to the invention. To date, the Hum4 VL has only been reported...

...DH)
segment and the heavy chain joining (JH) segment of the composite Hum4 VL, VH **antibody** are not critical to the oresent invention, Obviously, human and murine DH and jH gene...

...utilizing CC46 VH1 CC49 VH,
CC83 VH and CC92 VH9 the composite Hum4 VL9 VH **antibody** will be designed to utilize the DH and JH segments which naturally associated with those...cloning procedure (see, Horton etal., supra).

In a specific embodiment the composite Hum4 VL, VH **antibody** will be a "composite Hum4 VL, VHaTAG **antibody** ", means an **antibody** or immunoreactive fragment thereof which is characterized by having at least a portion of the...
...VHaTAG9 and
the nucleotide sequences encoding the VH Of the CC46, CC49, CC83 and CC92 **antibodies** , respectively. Figure 3 shows the corresponding amino acid sequences Of VHaTAG, CC46 VH1 CC49 VH...

...Of VHaTAG, CC46 VH, CC49 VH9 CC83 VH and CC92 VH shows that those CC. **antibodies** are derived from VHaTAG. Somatic mutations Occurring during productive rearrangement of the VH derived from...

...VL germline genes have been provided herein, the present invention is intended to include other **antibody** genes which are productively rearranged from the VHaTAG germline gene. Other **antibodies** encoded by DNA derived from VHaTAG may be identified by using a hybridization probe made...

...to the
5' end of the downstream gene.

The CDR from the variable region of **antibodies** derived from VHaTAG may be grafted onto the FR of selected VH9 ises, FR of a human **antibody** (see E.PO 0 239 400 to Winter). For example, the cell line, B17X2.

expresses an **antibody** utilizing a variable light chain encoded by a gene derived from Hum4 VL and a and is not specific for TAG
SUBSTITUTE SHEET
However, consensus sequences of **antibody** derived from the CDR1 Of VHaTAG (amino acid residues 31 to 35 of Figure 3...

...replaced by any DH and JH
sequence which does not affect the binding of the **antibody** for TAG-72 but, specifically, may -be replaced by the CDR3 of an **antibody** having its VH derived from VHaTAG, e.g., CC46, CC49, CC83 and CC92. ExeMDlary techniques for such reDiacement are set forth in Horton etal., supra.

The CH domains of **immunoglobulin** heavy chain derived from VHaTAG genesq for example may be changed to a human sequence...

...complete: or shortened human isatypes, i,e,, !gG (e.g,, IgGj, IgG21 IgG3, and IgGO, **IgA** (e.g., IgAl and IgA2), IgD, IgE9 **IgM** , as well as the various allotypes of the individual groups (see Kabat etal. (1991), supra...

...include
screening of combinatorial libraries Of VL-VH combinations using an Fab or single chain **antibody**
SUBSTITUTE SHEET
(SCFV) format expressed on the surfaces of i'd phage (Clackson, et al...

...However, according
to the teachings set forth herein, it is now DOSSible to clone SCFV **antibodies** in E.coli, and express the SCFVs as secreted soluble proteins. SCFV proteins produced in...

...cells (source cells) from
vertebrates in any one of various stages of age, health and **immune** response.
Cells coding for the ...blood and hybridomas) from an animal exoosed to TAG-72 may be probed for selected **antibody** producing B cells. Variabil-'Lty among B cells 30 derived from a common germline gene...

...Soc,, 85:2149-2154). Heavy and
light chains may be combined invitro to a-ain **antibody** activity (see Edelman, etal. (1963), Proc, Natl. Acad, Sci. USA, 50:753).

The present invention...

...variable
region. Nucleic acids coding for VHaTAG-coding homologs can be derived from cells producing **IgA** , !gD, IgEq **IgG** or **IgM** , most preferably from **IgM** and **IgG** , producing cells.

The VHaTAG-coding DNA homologs may be produced by primer extension, The term "...155: 335-350; and PCR Technology, Erlich (ed.) (1989). ILICR amplification of the mRNA from **antibody** -producing cells is set forth in Orlandi etal. (1989), Proc. Natl. Acad, Sci,, USA, 86...provide amplifi
cation and/or expression of a composite Hum4 VL, VHaTAG homolog single chain **antibody** , Transformation of

appropriate cell hosts with a recombinant DNA molecule of the present invention is...

...the endogenous heavy chain by well known methods; in this way, glycosylation patterns of the **antibody** produced would be human and not non-human derived,
Successfully transformed cells, i.e., cells containing a gene encoding a composite Hum4 VL9 VHaTAG homolog single chain **antibody** operatively linked to a vector, can be identified by any suitable well known technique for...assays are those where the binding of the composite Hum4 VL9 VHaTAG homolog single chain **antibody** to TAG-72 Droduces a detectable signal, either directly or indirectly, Screening for productive Hum4...

...DNA sequences encoding the light chain and heavy chain of the composite Hum4 VLs VH **antibody** may be inserted into separate expression vehicles, or into the same expression vehicle, When coexpressed...

...e. be complementary co) a conserved region within the J-region or constant region of **immunoglobulin** light chain genes and the 11ke. Second Drimers become Dart of the coding (plus) strand...

...U
SUBSTITUTE SHEET
thus Dossible to use an iterative process to define yet further, composite **antibodies** , using later generation VHaTAG-coding DNA homologs and Hum4 VL-coding DNA homologs.

The present invention further contemDiates genetically modifying the **antibody** variable and constant regions to include effectively homologous variable region and constant region amino acid...Princioles and Aoolications for DNA
Amolification, Erlich, (ed.) (1989); and Horton etal. supra).

Further, the **antibodies** may have their constant region domain modified, ie,, the CL, CH1@ hinge,, CH2, CH3 and/or CH4 domains of an **antibody** polypeptide chain may be deleted, inserted or changed (see EPO 327 378 Al to Morrison...

...the mice, which yields a very high t-iter of homogeneous composite Hum4 VL9 VH **antibodiesq** and isolating the composite Hum4 VL9 VH **antibodies** by methods well known in the art (see Stramignoni. etal.

(1983), Intl. J. Cancer, 31...

...of which can provide up to about 50 mg/mL of composite Hum4 VL, VH **antibodiese** Usually, injection (preferably intraperitoneal) of about 106 to 107

histocompatible host cells into mice or...

...formation after a few weeks. It is possible to obtain the composite Hum4 VL? VH **antibodies** from a fermentation culture broth of procaryotic and eucaryotic cells, or from inclusion bodies of...

...see Buckholz and Gleeson (1991), BIO/TECHNOLOGY, 9:1067 1072. The composite Hum4 VLP VH **antibodies** can then be collected and processed by well-known methods (see generally, Immunological Methods, vols, ...ed, Weir, D., (1978) Blackwell Scientific Publications, St. Louis, MO.)
The composite Hum4 VL, VH **antibodies** can then be stored in various buffer solutions such as phosphate buffered saline (PBS), which aives a generally stable 0
antibody solution for further use.

Uses
The composite Hum4 VL, VH **antibodies** orovide unique benefits for use in a variety of cancer T
treatments. Ln addition to...
...fibroblasts, endothelial cells, or epithelial cells in the major organs, the composite Hum4 VL, VH **antibodies** may be used to greatly minimize or eliminate ANHA responses thereto.

Moreover, TAG-72 contains...

...epitopes and thus it may be desirable to administer several different composite Hum4 VL, VH **antibodies** which utilize a variety Of VH in combination with Hum4 VL
Specifically, the composite Hum4 VLi VH **antibodies** are useful for, but not limited to, invivo and lavitro uses in diagnostics, therapy, imaging and biosensors,
The composite Hum4 VL7 VH **antibodies** may be incorporated into a pharmaceutically acceptable, non -toxic, sterile carrier. Injectable compositions of the...

...useful suspending agents.

Methods of preparing and administering conjugates of the composite Hum4 VL9 VH **antibody** , and a therapeutic agent are well known to or readily determined, Moreover, suitable dosages will...

...employed and are well known or readily determined.

Conjugates of a composite Hum4 VL9 VH **antibody** and an imaging marker may be administered in a pharma ceutically effective amount for the...appropriate detection means, Administration and detection of the conjugates of 'Che comoosite Hum4 VL, VH **antibody** and an imaging

marker, as well as methods of conjugati,ng the composite Hum4 VLi VH **antibody** to the imagin.g marker are accomplished by methods readily known or readily determined, The...

...0.1 mg to 200 mg of the conjugate of the composite Hum4 V'L **antibody** and imaging marker per patient.

Examples of imaging markers which can be conjugated to the composite Hum4 VL **antibody** are well known and include substances which can be detected by diagnostic imaging using a...

...t-hat expresses TAG-72, A I'pharmaceutically effective amount" of the composite Hum4 VL **antibody** means '%','he amount of said **antibody** (whether unconjugated. i,e., a naked **antibody** , or conjugated to a therapeutic agent) in the pharmaceutical comoosition should be sufficient to achieve effective binding to TAG
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PC'r/AU91/00583
Exemplary naked **antibody** therapy includes, for example, administering heterobifunctional composite Hum4 VL9 VH **antibodies** coupled or combined with another **antibody** so that the complex binds both to the carcinoma and effector cells, e.g., killer cells such as T cells, or monocytes. In this method, the composite Hum4 VL **antibody** -therapeutic agent conjugate can be delivered to the carcinoma site thereby directly exposing the carcinoma tissue to the therapeutic agent, Alter natively, naked **antibody** therapy is possible in which **antibody** dependent cellular cytoxicity or complement dependent cytotoxicity is mediated by the composite Hum4 VL **antibody** *
Examples of the **antibody** -therapeutic agent conjugates which can be used in therapy include **antibodies** coupled to radionuclides, such as 131I, 90y@ 105RhJ 47SC, 67Cu. 212Bi, 21'At, 67Ga, 1251...

...such as ricin.

Methods of preparing and administering conjugates of the composite Hum4 VL, VH **antibodies** and a t erapeutic agent are well known or readily determined.

The pharmaceutical composit!.on...the therapeutic agent employed and are well known or readily determined.

Composite Hum4 VL, VH **antibodies** , and parti cularly composite Hum4 VT,
La VH single chain **antibodies**
thereof, are Particularlv suitable for radioimmunaguided
SUBSTITUTE SHEET
surgery (RIGS), In RIGS, an **antibody** labeled with an imaging marker is injected into a patient having a tumor

that expresses TAG The **antibody** localizes to the tumor and is detected by a hand-held gamma detecting probe (GDP...

...The relatively small size and human character of the composite Hum4 VL, VH single chain **antibodies** will. accelerate whole body clearance and thus reduce the waiting period after injection before surgery can be effectively initiated.

Administration and detection of the composite Hum4 VLf VH **antibody** -imaging marker conjugate may be acCOMDlished by methods well-known or readily determined.

The dosage...

...patient, but generally a one time dosage of about 0.1 to 200 mg of **antibody** -marker conjugate per patient is administered.

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EXAMPLES
The following nonlimiting examples are merely for illustration of the construction and expression of composite Hum4 VL, VH **antibodies** . All temperatures not otherwise indicated are Centigrade. All percents not otherwise indicated are by weight...

...Figure 7), following the procedures set forth in EPO 0 365 997.

DNA encoding an **antibody** light chain was isolated from a sample of blood from a human following the protocol...next day with 1 percent BSA. Supernatant samples to be tested for anti-TAG-72 **antibody** were added to the washed wells and incubated for between 1 and 2 hours at 37 IC.

Alkaline phosphatase labeled goat ant;i-human **IgG** (diluted 1:250) (Southern Biotech Associates, Birmingham. AL) was used as the probe **antibody** .

Incubation was for 1 hour. The substrate used was D nitrophenylphosohate, Color development was terminated...

...of half a cell oer well (nominally 50 cells) to get Dure monoclonal cell 'Lines, **Antibody** producing cell lines were frozen down In media containing 10 percent DMSO, Two cell 11ines...of the cell line MPl-44H was grown at 37'C for 5 days for **antibody** production. The culture supernatant was obtained free of cells by centrifugation and filtration through a...

...filter apparatus, The clarified supernatant was passed over a Protein A cartridge (Nygene, New York). **Immunoglobulin** was eluted using 0.1 M sodium citrate buffer pH 30. The pH of the eluting fractions containing the **antibody** was raised to neutrality by the

addition of Tris base, pH 9.0, The **antibody** -containing fractions were concentrated and oassed over a Pharmacia Superose 12 HR 10/30 gel...

...focusing further demonstrated the purity of MP1-44H. The biological performance of the human composite **antibody** , MP1-44H. was evaluated by comparing immunohistochemistry results with two other anti-TAG-72 antibdoies...

...HB 9884), Sections of human colorectal tumor embedded in oaraffin were tested with the three **antibodies** by methods familiar to those skilled in this art. All three **antibodies** gave roughly equivalent binding recognition of the tumor antigen present on the tumor tissue samole.

no
A further test of IChe affinity and biological integrity of the human composite **antibody** MP1-44H was a w
competition assay, based on cross-competing radioiodine -labeled versions of the **antibody** with CC49 and Ch44 in all combinations. From the data shown in Figure 20, it SUBSTITUTE SHEET
is apparent that the affinity of all 3 **antibodies** is equivalent and can bind effectively to tumor antigen.

MP1-44H (ATCC HB 10426) andmpl...

...will be prOMDtly replaced on notification with viable replacement cell lines.

Examnle 2
Single-chain **antibodies** consist of a VL7 VH and a peptide linker joining the VL and VH domains to produce SCFVs. A single chain **antibody** , SCFV1, was constructed to have the Hum4 VL as V Domain 1 and CC49 VH...SCFV1 had TAG-72 binding activity, The SCM protein has been detected by a standard **Western** protocol (see Towbin etaL (1979), Proc.

Natl. Acad, Sci., U.S.A., 76:4350-4354), The detecting agent was biotinylated FAID14 (ATCC No. CRL 10256), an anti-idiotypic monoclonal **antibody** prepared from mice that had been immunized with CC49. A band was visualized that had...designated Fragment C. pRW 83 was isolated from E. coli strain GM161, which is DNA **methylase** minus or **dam** -.

Plasmid pSCFV 31 (see Figure 24) was created with a three part ligation Fragments A...oligonucleotides shown below. The linker UNIHOPE is based on 205C SCA" linker (see Whitlow, (1990) **Antibody** EnRineerinz: New Technology and ADolication TMD!ications TBC USA SUBSTITUTE SHEET
Conferences Inc, MA), but...

...P-lactamase penP
promoter, pectate lyase pelB signal sequence and the penP terminator region. Different **immunoglobulin** light chain variable regions can be inserted in the Nco I-Hind III restriction sites, different SCFV linkers can be inserted in the Hind III-Xho I sites and different **immunoglobulin** heavy chain variable regions can be inserted in the Xho I-Nhe I sites.

E...SUBSTITUTE SHEET
Discovery of Hum4 VL-VH combinations that compece with known prototype TAG-binding **antibodies** or mimetics.
oSCFVUHH Xho IINhe I
Vector DNA Fragment
(CC49 VH removed)
or pATDFLAG XhoI...24-96 hours.

bind to TAG.

Process hydrophobic membrane using a prototype biotinylated TAG-competing **antibody** , e.g. B72 CC49r CC83 or biotinylated competing peptide or mimetic. Use assay streptavidin conjugated...

...Determine normai:tumor tissue binding profile by immunohistocnemistry.

Utilize Hum4 VE and VH in preferred **antibody** formats e.g. whole **Ig** (IgGle IgEr **IgM** etc.) Fab or F(ab')2 fragment, or SCFV.

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Isolating total RNA frcm...ambient temperature from I hour with gentle shaking. After incubation, the membranes are blocked with **Western** blocking solution (25 mM Tris, 0.15 M NaCl, pH 7.6; 1% BSA) for...

...removed from the agar surface. The TAG-72 membrane is placed in 20 ml of **Western** **antibody** buffer (TBS in 0.05% Tween-20, cat# F-1379, Sigma Chemical Co.; 1% BSA, cat#3203, Biocell Laboratories) containing 0.2 ng of CC49-Biotin probe **antibody** , The bacterial membranes are replaced on the agar surface in their original orientation and set...

...at 300 rom. StreDtavidin alkaline phosphatase (cat# 7100-04, Southern Biotechnology Associates) is added to **Western** **antibody** buffer to produce a 0.1100 solution, The TAG-72 membranes are each immersed in...

...incubation, the membranes are washed as Dreviously described. A final wash is then performed using **Western** alkaline phosphate buffer (8.4 g NaCO3, 0.203 g MgC12-H9@09 pH 9.8), for 2 minutes at 200 rpm at ambient temperature, To develop the membranes, 5 **Western** blue stabilized substrate (cat# S384B, Promega)

is added to each membrane surface. After 30 minutes...

...according to the schematic, supra, exceDt for the following: at the assay step, IBI MII **antibody** is used as a probe to detect any Hum4 VL - VH SCFV combinations '@5 that...as the 3' end oligonucleotide. The resulting DNA and amino acid sequences of this SCFV **antibody** , with the FLAG peptide at the C-terminus, --is shown in Figure 30. The PCR...cells (Stratagene, La Jolla) is achieved following the supplier's protocol.

IBI MII Anti-FLAG **Antibodv** Plate Assay
The first three steps, preparation of rlr'AG coated membranes, plating of bacteria...

...membranes are washed with TTBS three times at 250 rpm for four minutes, The MII **antibody** (cat# IB13010, International Biotechnologies, inc.) is then diluted with TBS to a concentration ranging from 10.85 jig/ml to 0.03 pg/ml. Ten millilters of the diluted **antibody** are added to each membrane. The membranes are then incubated for 1 hour at ambient temperatures and shaken on a rotary shaker at TO rom, After incubation, the MII **antibody** is removed and the membranes are washed three times at 250 rpm and ambient temperatures...for binding to a specific epitope by using the competition assay, supra, and a competing **antibody** or mimetic, if desired.

The present invention is not to be limited in scope by...

Claim

1 A composite Hum4 VL, VH **antibody** having binding affinity for TAG-72 comprising A. a light chain having a variable region...

...dimensional structure having the ability to bind TAG-72*

2 The composite Hum4 VL, VH **antibody** of Claim 1. wherein the VL is further encoded by a. human J gene segment.

3 The composite Hum4 VL, VH **antibody** according to Claim 1, wherein the VH is encoded by a DNA seauence comorising a...

...to the VT .jaTAG germline gene (VjjcLTAG). SUBSTITUTE SHEET

4 The comDosite Hum4 VL9 VH **antibody** of Claim 1 wherein the VH is further encoded by an animal D gene segment and an animal J gene segment.

5 The composite Hum4 VL, VH **antibody** -of

Claim 1, wherein the variable region is derived from the variable regions of CC46, CC49, CC83 or CC92,

6 The composite Hum4 VL9 VH **antibody** of Claim 1, wherein the VH comprises (1) complementarity diversity regions (CDR) being encoded by...

...to
the CDR segments, encoded by a human genes.

7 The composite Hum4 VL9 VH **antibody** of Claim 1, wherein the light chain further comprises at least a portion of a...

...least a portion of a
animal constant region (CH)

8 The composite Hum4 VL@ VH **antibody** of is IgG1.4, **IgM** , igAl, IgA2, IgD
Claim 6. wherein the CH
or IgE.

9 The comoosite Hum4 VL, VH **antibody** of Claim 7, wherein C is kappa or lambda.
L
10* A composite Hum4 VL9 VH single chain **antibody** or immunoreactive fragment thereof comprising (a) a light chain having a variable region (VL), said...

...VL,
wherein the polypeptide linker properly folds the VH and VL into a single chain **antibody** which is capable of forming a three dimensional structure having the ability to bind TAG 72,
Ile A composite Hum4 VL1 VH **antibody** conjugate comprising the composite Hum4 VLI VH **antibody** of Claims I through 10 conjugated to an imaging marker or a therapeutic agent.

12 The composite Hum4 VLF VH **antibody** conjugate of Claim 11, wherein the imaging marker is selected from the group consisting of...

...153SM,
67CU, 67Ga, 166RO, 177Lu, 186Re, 18SRe. and 99mTc,
13* The composite Hum4 VL, VH **antibody** conjugate of claim 11, wherein the therapeutic agent is a drug or biological response modifier, radionuclide, or toxin.

14 The composite Hum4 VL1 VH **antibody** conjugate of claim 13, wherein the drug is methotrexate, adriamycin or interferon.
15* The composite Hum4 VLf VH **antibody** conjugate of Claim 13, wherein the radionuclide is 131J, 90y, 105Rhl 47SCf 67CUI 212Bir 211At...

...99MTCI
153SM, 123I or IIIIn.

16 A composition comprising the composite Hum4 VL1 VH
25 **antibody** of Claim I in a pharmaceutically acceptable, non toxic, sterile carrier,
SU13STITUTE SHEET
PCf/AU91/00583o A composition comprising the composite Hum4 VL, VH **antibodyof** Claim 12 in a pharmaceutically acceotable, non-toxic, sterile carrier,
18* A composition comprising the composite Hum4 VLY VH **antibody** of Claim 13 in a pharmaceutically acceptable, non-toxic, sterile carrier.

19 A method for...

...a human.
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25 A cell capable of eXDressing the composite Hum4 VL, VH **antibody** or immunoreactive fragment thereof of Claim 1, said cell being transformed with
(A) a first...

...biologically functional expression vector.

27 A process for producing a composite Hum4 2G VL, VH **antibody** comprising at least the variable domains of the **antibody** heavy and light chains, in a single host cell, comprising the steps of:
As transforming...present in at least one vector.

29 The process according to Claim 28 wherein the **antibody** heavy and light chains of the composite Hum4 VL, VH **antibody** are expressed in the host cell are secreted therefrom as an immunologically functional **antibody** molecule or **antibody** fragment.

30 The process of Claim 27, wherein the second DNA sequence encodes the VH Of CC469 CC49@ CC83 or CC92,

31 A process for preparing an **antibody** or **antibody** fragment conjugate which comprises contacting:
the composite Hum4 VL9 VH **antibody** of Claim 1 with an imaging marker or therapeutic agent,

32 The process of Claim...
...wherein the
therapeutic agent is a radionuclide, drug or biological response modifier, toxin or another **antibody** ,
@0
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20/3,KWIC/71 (Item 8 from file: 654)
DIALOG(R)File 654:US Pat.Full.
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3926426
Derwent Accession: 1995-185787
Utility
C/ **Methods of analysis and manipulating of DNA utilizing mismatch repair systems**
Inventor: Modrich, Paul L., Chapel Hill, NC
Su, Shin-San, Newton, MA
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Lahue, Robert S., Northboro, MA
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Duke University (Code: 25202)
Examiner: Jones, W. Gary (Art Unit: 187)
Assistant Examiner: Rees, Dianne
Law Firm: Lyon & Lyon LLP

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Main Patent	US 5702894	A	19971230	US 95460663	19950602
Continuation	US 5556750	A		US 93145837	19931101
Continuation	Abandoned			US 89350983	19890512
CIP	Abandoned			US 932529	19930111

Fulltext Word Count: 21706

Description of the Invention:

...membranous nitrocellulose filter, detection of the DNA:protein complex further includes the step wherein an **antibody** specific for the base mispair recognition protein is employed, the base mispair recognition protein is... **Antibodies** specific for a DNA mispair recognition protein can be prepared by standard immunological techniques known...

...Other suitable analytical methods for detecting the DNA protein complex include immunodetection methods using an **antibody** specific for the base mispair recognition protein. For example, **antibodies** specific for the E. coli MutS protein have been prepared. Accordingly, one immunodetection method for...

...DNA:protein complexes from DNA that does not form such complexes by immunoprecipitation with an **antibody** specific for MutS protein, and detecting the DNA in the precipitate. According to the practice... otherwise GATC unmodified molecules is desired, this can be accomplished by use of E. coli **Dam methylase** as is well known in the art. Symmetrically methylated DNA prepared by use of this...incised with MutH protein as described in the legend to FIG. 4. When present, rabbit **antiserum** to helicase II or preimmune serum (5 [mu]g protein) was incubated at 0[degree...

...the cofactor was then added and the assay was performed as above. Although not shown, **antiserum** inhibition was reversed by the subsequent addition of more helicase II. With the exception of...

...absence of the added DNA helicase II (Table 2). Nevertheless, the reaction was abolished by **antiserum** to homogeneous helicase II,

suggesting a requirement for this activity and that it might be...other methods of the invention; e.g., altered electrophoretic mobility, or detection by use of **antibodies** .

20/3,KWIC/90 (Item 27 from file: 654)

DIALOG(R)File 654:US Pat.Full.
(c) Format only 2005 The Dialog Corp. All rts. reserv.

3496330 **IMAGE Available
Derwent Accession: 1994-159179
Utility
REASSIGNED
C/ **Cloned streptococcal genes encoding protein G and their use to construct recombinant microorganisms to produce protein G**
; **GENETIC ENGINEERING WITH** IMMUNOGLOBULINS **AND PROTEINS**
Inventor: Fahnestock, Stephen R., Olney, MD
Assignee: Pharmacia LKB Biotechnology AB(03), SE
Pharmacia LKB Bioltechnology AB SE (Code: 27361)
Examiner: Nucker, Christine M. (Art Unit: 183)
Assistant Examiner: Sidberry, H.
Law Firm: Sterne, Kessler, Goldstein & Fox

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5312901	A	19940517	US 92871539	19920421
Division	Pending			US 90540169	19900619
Division	US 4956296	A		US 88209236	19880620
CIP	US 5082773	A		US 8763959	19870619
CIP	Abandoned			US 86854887	19860423
CIP	Abandoned			US 86829354	19860214

Fulltext Word Count: 15408

Abstract:
Protein G variants are disclosed. These protein G variants have the **immunoglobulin** binding properties of protein G. Also disclosed is the preparation of the protein G variants...

Summary of the Invention:
...the biosynthesis of Streptococcus Protein G, cloned genes which encode protein G variants having the **immunoglobulin** binding properties of protein G, and the use of organisms transformed with the cloned genes ...

...growing interest in recent years in bacterial F[sub]c receptors, molecules that bind to **antibodies** through a nonimmune mechanism. This binding is not to the antigen recognition site, which is located in the F[sub]ab portion of the **antibody** molecule, but to the F[sub]c portion of the **antibody** . The F[sub]c region is common to many types of **antibodies** , thus bacterial F[sub]c receptors can bind to many types of **antibodies** . This property makes bacterial F[sub]c receptors useful in a number of immunochemical applications...

...receptors have a number of useful or potentially useful applications,

primarily in the detection of **antibodies** , the purification of **antibodies** and the treatment of diseases. The detection of **antibodies** is required in several phases of laboratory research in immunology, including the screening of hybridoma clones for the secretion of specific monoclonal **antibodies** , the measurement of the **immune** response of an immunized animal, and the quantitation of antigens by competitive binding assays. Methods for detecting **antibodies** using bacterial F[sub]c receptors have been found to be more sensitive and less...

...F[sub]c receptors also are useful in purifying **antibodies** to be sued in the purification of protein drugs and as therapeutics. Although a number...

...Protein A of Staphylococcus aureus, which binds to the constant F[sub]c domain of **immunoglobulin** **IgG** . Other bacterial F[sub]c receptors also have been identified. One of these is known...

...G has several important advantages. For example, Protein G binds to all subclasses of human **IgG** , whereas Protein A does not bind to the IgG3 subclass [Reid, K. J. et al. J. Immunol. 132:3098-3102 (1984)]. Protein G also is specific for **IgG** and does not cross-react with human **antibodies** of type **IgA** and **IgM** as Protein A does. [Myhre, E. B. and Kronvall, G. " **Immunoglobulin** Specificities of Defined Types of Streptococcal **Ig** Receptors " In: Basic Concepts of Streptococci and Streptococcal Diseases; J. E. Holm and P. Christensen...

...not at all. These include bovine, ovine, and caprine IgGl and several subclasses of equine **IgG** (Reis, K. J. et al., supra). Protein G also has been found superior to Protein A in binding to several subclasses of murine monoclonal **antibodies** [Bjorck, L. and Kronvall, G. J. Immunol. 133:969-974 (1984)]. For these reasons, Protein...present invention provides a cloned gene encoding an F[sub]c receptor protein having the **IgG** binding properties of Protein G. The gene is derived from Streptococcus Sp., Lancefield Group G...

...further provides for the production, using recombinant vectors, of Protein G variants, which have the **immunoglobulin** binding properties of Protein G, the Protein G variant polypeptides containing one or more amino acid sequences which correspond to the **Ig** binding sites of Protein G and which exhibit the **IgG** -binding characteristics of Protein G.

Description of the Drawings:

...the cloned protein G gene and the repeating structure of its protein product responsible for **IgG** -binding...

Description of the Invention:

...contain portions of the respective sequences corresponding to B1 and B2 and which retain the **immunoglobulin** binding properties of Protein G. Such a hybrid sequence is shown in FIG. 9 and...

...282 of B1. Thus, it is intended that all such hybrid sequences which retain the **immunoglobulin** binding properties of Protein G are within the scope of this invention...

...may be inserted into various multicopy expression vectors to give enhanced levels of these valuable **IgG** -binding proteins in cultured E. coli cells transformed with the recombinant expression vectors.

Production of...

...Streptococcal strains that produce Protein g. This may be done by assaying various strains of **IgG** binding activity using any suitable immunoassay technique. A technique used by the Applicant is the colony immunoassay described in detailed in the example section below. Strains found to have **IgG** -binding activity are next tested for the ability to bind IgG3 as well as unfractionated **IgG** , since the ability to bind IgG3 is a desired property associated with Protein G. A hemagglutination assay using red blood cells coated wither with IgG3 or with unfractionated **IgG** (describe din detail in the examples below) is a convenient method for identifying Protein G...

...78: 471-490 (1977)], may be used as a control, since Protein A binds unfractionated **IgG** bu not IgG3...functionally active portions of Protein G was localized to a repeating structure by examining the **IgG** -binding activity of protein produced by E. coli strains carrying modified forms of the cloned...

...of Protein G (Protein G variants) and to the protein so produced which has the **immunoglobulin** binding properties of Protein G. The details of the identification and isolation of the gene...

...Also within the scope of this invention are Protein G variants having the **immunoglobulin** -binding properties of Protein G, further having deletions or substitutions of amino acids or additional...combinations constructed in this way retain sequences encoding the B domains, and therefore retain the **IgG** -binding activity of Protein G. Additionally, it will be recognized that the SmaI-BamHI fragment...

...may be purified from the cell lysate using such standard procedures as adsorption to immobilized **immunoglobulin** , as described by Sjoquist, .S. Pat. NO. 3,850,798 (1974), ion-exchange or gel...strains were derived from the clinical isolates. Each strain was assayed for ability to bind **IgG** using the following colony immunoassay procedure. The strains were streaked on L-Broth-agar plates...

...The nitrocellulose sheets then were removed from the plates, and **IgG** -binding proteins were detected on the sheets using an immunochemical procedure, as follows. The sheets...

...and 0.15 M Nacl) to block nitrocellulose sites to minimize non-specific binding of **antibodies** to the nitrocellulose in subsequent steps. The sheets then were treated with normal rabbit serum...

...for 1 hours at 23[degree(s)] C., followed by peroxidase-conjugated goat anti-rabbit **IgG** (similarly diluted), and, finally, with 4-chloro-1-naphthol (0.6 mg/ml) and hydrogen...

...Tris-saline between incubation steps. Blue spots on the nitrocellulose sheet indicate the presence of **IgG** -binding protein, and the blue areas correspond to microbial colonies which produced the **IgG** -binding protein ...

...Nine of the strains were positive, i.e. were found to bind **IgG** , although to varying degrees. Several of the strains were next tested for ability to bind...

...following hemagglutination assay. Sheep red blood cells (RBC) (Cappel

Laboratories, Malvern, Penn.) were coated with **immunoglobulin** essentially as described by Adler and Adler [Meth. Enzymol. 70:455-466 (1980)]. RBC were...

...centrifugation and resuspended in PBS containing, at 0.2 mg/ml, either (a) total human **immunoglobulin** G (available from Sigma Chemical Co; St. Louis, Mo.), (b) IgG3 myeloma protein or (c...

...group G Streptococcal strains agglutinated IgG3-coated erythrocytes as efficiently as erythrocytes coated with unfractionated **IgG** , which is expected for Protein G-producing strains. In contrast, Staphylococcus aureus Cowan I cells...

...cultures of the Streptococcus isolates and the isolates appeared to have differing localization of the **IgG** -binding activity. In some strains the activity appeared to be predominantly cell-bound, ...culture supernatant, and some strains were intermediate. Three stains, which had differing localization of the **IgG** -binding activity, were chosen as sources of DNA for cloning the Protein G gene...The nitrocellulose sheets then were removed from the plates, and **IgG** -binding proteins were detected on the sheets using the immunochemical procedure described above. The sheets...

...w/v in tris-saline) to block introcellulose sites to minimize non-specific binding of **antibodies** to the nitrocellulose in subsequent steps. The sheets the were treated with normal rabbit serum...

...for 1 hour at 23[degree(s)] C. followed by peroxidase-conjugated goat anti-rabbit **IgG** (diluted similarly), and, finally, with 4-chloro-1-naphthol (0.6 mg/ml) and hydrogen...SDS ges as described by Studier, op cit., to separate the proteins. A standard electrophoretic (**Western** Blotting) technique was used to transfer the protein bands from the gel to nitrocellulose paper...

...was subsequently incubated (in sequence) with BSA, normal rabbit serum, peroxidase-conjugated goat anti-rabbit **IgG** , and 4-chloro-1-naphthol plus H[sub]2 O[sub]2 (the same nitro...

...treatment as the immunochemical procedure described above). Both strains were found to produce the same **IgG** -binding protein bands with mobilities corresponding to molecular weights between approximately 90,000 to approximately...

...was subcloned into pGX1066, and the resulting recombinant plasmid (pGX4547) was transformed into E. coli. **Western** blotting of the proteins produced by this transformant (E. coli GX7841) was done as described above, and the same **IgG** -binding protein bands were present including the predominant 57,000 band. The transformant was also...

...extract from a Protein A-producing E. coli strain agglutinated the erythrocytes coated with unfractionated **IgG** , but not those coated with IgG3 or uncoated erythrocytes. A control E. coli strain which...These results demonstrate that E. coli strains GX7841, GX7820, and GX7823 produce **IgG** -binding protein having the properties which are characteristics of Protein G...

...protein, are included int eh present invention as long as the protein retains the desired **IgG** -binding properties of Protein G, described above. These variations in amino acid sequence may be...

...Identification of the Portions of the Protein G Molecule Responsible for the **IgG** -binding Activity...

...By examining the **IgG** binding activity of protein produced by E. coli strains carrying deleted and modified forms of...infected from several plaques, and the same infected cells were assayed for the production of **IgG** -binding protein by colony immunoassay. Several clones were found by analysis of RF DNA with...

...415 bp PstI fragments indicated in FIGS. 5 and 6. These clones produced no active **IgG** -binding protein, as indicated by colony immunoassay. The truncated protein produced by these clones would...

...Polyacrylamide gel electrophoretic analysis revealed that E. coli bearing this DNA produced a protein with **IgG** -binding activity of approximately the expected size [Fahnestock, et al., J. Bacteriol. 167:870-880...

...that the presence of the B repeated structure is a necessary and sufficient condition for **IgG** -binding activity of protein G. It was therefore concluded that the B repeating structure was the locus of **IgG** -binding activity in the molecule...Strain GX8408 was shown to produce a protein with the **IgG** -binding activity of protein G. This strain, where sequences distal to the B2 sequences are...

...bands were transferred to nitrocellulose and stained immunochemically as described in Example I. Material with **IgG** -binding activity was found in both the culture supernatant and cell-associated fractions...the transformed cells were plated on colony immunoassay plates and assayed for the production of **immunoglobulin** -binding protein as described in Example I. A positive colony was identified. Plasmid DNA isolated...

...from the active B repeats (Example III) have been deleted. Such proteins, which exhibit the **immunoglobulin** binding activity of protein G, have enhanced stability toward proteolysis...by analysis similar to that outlined in Example IV above, to produce protein with the **immunoglobulin** binding activity of Protein G, and this protein could be detected in both the extracellular...designated GX8455 (pGX4597) and GX8457 (pGX4599). Both strains were found to synthesize protein with the **immunoglobulin** binding activity of Protein G, and in both cases this protein was detected in both...selecting for ampicillin resistance at 30[degree(s)] C. Transformants were screened for production of **IgG** binding protein at 42[degree(s)] C., as described in Example VII. One positive transformant...was shown to produce a protein of the expected size with binding activity for human **IgG** . This protein has been designated protein G variant Type 1. The amino acid sequence of...Both GX8464 and GX8465 were shown to produce protein variants with the **immunoglobulin** binding activity of Protein G. In addition, both strains exhibited enhance production and produced about...Plasmid pGX4599 was used to transform E. coli GM272, a strain which lacks the **dam methylase** . Plasmid DNA obtained from one such transformant was digested with restriction endonuclease ClaI. Because of...to produce a protein of the expected size with the ability to bind to human **IgG** . This protein G variant contains a single **IgG** binding sequence B2 (from GX7809 protein G), the adjacent proline-rich region, and the "C...

...such that significant quantities of a 420 bp fragment derived from the region encoding the **IgG** -binding domains of protein G were present. This 420 bp PstI fragment was isolated following...

...in pGX5204. The strain containing pGX5247 was designated GX8825 and was shown to produce an **IgG** -binding protein of the expected size. The predicted structure of this protein (type 6) is...Plasmid pGX4595 was used to transform E. coli GM272, a strain which lacks the **dam methylase** . Plasmid DNA obtained from one such transformant was digested with restriction endonuclease ClaI. Because of...to produce a protein of the expected size with the ability to bind to human **IgG** . The predicted structure of this protein is as follows...

...This structure contains a single **IgG** binding sequence B2 (from GX7809 protein G) plus several amino acid residues derived from the...the desired structure (pGX5255) was designated strain GX8833. This strain was shown to produce an **IgG** -binding protein of the expected size, with approximately one Cys residue per molecule. The structure...Plasmid pGX4599 was used to transform E. coli GM272, a strain which lacks the **dam methylase** . Plasmid DNA obtained from one such transformant was partially digested with restriction endonuclease ClaI under...

Exemplary or Independent Claim(s):

1. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence:

Non-exemplary or Dependent Claim(s):

2. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence...

...3. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence...

...4. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence...

...5. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence...

...6. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence...

...7. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence...

...8. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence...

...9. A protein G variant having the **immunoglobulin** -binding properties of protein G, the polypeptide consisting essentially of the amino acid sequence...

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3452812
Derwent Accession: 1994-006635
Utility
C/ **Vaccines for the protection of animals against theileria infection ; A PURE AND ANTIGENIC POLYPEPTIDE FOR CATTLE IMMUNITY**
Inventor: Musoke, Anthony J., Kampala, UG
Nene, Vish, Cambridge, GB England
Iams, Keith, Pittsburgh, PA
Nantulya, Vinand M., Mbale, UG
Assignee: International Laboratory for Research on Animal Diseases(03), Nairobi, KE
International Laboratory for Research on Animal Diseases KE (Code: 32513)
Examiner: Furman, Keith C. (Art Unit: 184)
Law Firm: Townsend and Townsend

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5273744	A	19931228	US 92835043	19920211
Continuation	Abandoned			US 89365999	19890614

Fulltext Word Count: 10350

Summary of the Invention:
...review of the disease can be found in Irvin A. D. and Morrison W. I. **Immune** Responses in Parasitic Infection: Immunology, immunopathology, and immunoprophylaxis Vol. III, Ed. E. J. L. Soulsby...

...a high rate of mortality; however, some animals recover from the infection and are subsequently **immune** to homologous challenge. At present, it is possible to induce immunity by infecting cattle with fever: correlation between monoclonal **antibody** profiles of Theileria parva isolates and cross immunity in vivo. Res. Vet. Sci. 35:341...

...It has been shown that animals which have developed immunity to T. parva exhibit **antibody** responses against proteins of the sporozoite stage (Musoke, A. J., et al., 1982, Bovine **immune** response to Theileria parva: neutralizing **antibodies** to sporozoites, Immunology 45:663-668) as well as a cell-mediated response against the...

...Eugui, E. M. and Emery, D. L., 1981, Genetically restricted cell-mediated cytotoxicity in cattle **immune** to Theileria parva. Nature 290, 251-254). There is evidence that **antibodies** raised against sporozoite antigens can block infectivity in vitro in a non-isolate specific manner...

...several Theileria parva strains. Immunology 52, 231-238; and Dobbelaere, D. A. E., 1984, Monoclonal **antibody** neutralizes the sporozoite stage of different Theileria parva isolates, Parasite Immunol. 6:361-370). However ...

...E. et al., 1985, Identification of a surface antigen on Theileria parva sporozoites by monoclonal **antibody** , Proc. Natl. Acad. Sci., U.S.A. 82:1771-1775; and Dobbelaere, D. A. E...

Description of the Invention:

...on the 67 kDa antigen of T. parva parva (Muguga) is conserved since one monoclonal **antibody** will in an in vitro assay neutralise sporozoites from different isolates of the parasites. The...

...that contains the gene encoding the 67 kDa antigen. Live vaccines will induce a potent **immune** response against T. parva, without the need for purification of the 67 kDa antigen. The...of the expressed antigen is achieved by methods known in the art as radioimmunoassays, or **Western** blotting techniques or immunoprecipitation. Purification from E. coli can be achieved following procedures described in...

...of the expressed antigen is achieved by methods known in the art such as radioimmunoassays, **Western** blotting or immunoprecipitation...

...attenuation of the strains render the bacteria avirulent but still capable of inducing a potent **immune** response after inoculation into cattle. An example of such a strain is the aro A...by DNA hybridization using cDNA encoding the 67 kDa antigen and by immunodetection techniques using **antibodies** specific for the expressed protein. Virus stocks may be prepared by infection of cells such...techniques to the lysates. The monitoring of the purification process can be accomplished by using **Western** blot techniques or radioimmunoassays...weeks later. Animals that have been previously exposed to Theileria parva or have received colostral **antibodies** from the mother may require booster injections. The booster injection is preferably timed to coincide...contaminating proteins is such that the vaccinated animal will not respond with significant levels of **antibodies** against said contaminants. Typically, the antigen preparation will be pure to at least 75%, preferably...

...in the amino acid sequence and carbohydrate side chains such that they appear to the **immune** system as functional equivalents for purposes of protection from Theileria infection. These non-natural derivatives...

...immunologically equivalent to) the naturally occurring epitopes. Such proteins would exhibit cross reactivity with the **antisera** produced against the natural 67 kDa antigen. These protein derivatives may include peptide fragments, amino...The phrase "Theileria sera" refers to blood serum containing **antibodies** reactive with native 67 kDa antigen...in from the N-terminal end (FIG. 1). Since BclI digestion is blocked by the **dam methylase** , plasmid 2 is grown in a **methylase** -deficient strain of E.coli such as NK5772. Plasmid 2 is prepared from NK5772 and... transformed into avirulent (aroA) Salmonella typhimurium and expression of the fusion protein is monitored by **Western** blotting...

...cured of the resident plasmids and tested for expression of the 67 kDa antigen by **Western** blotting. Confirmation of chromosomal integration is achieved by preparation of chromosomal DNA from recombinant strains...can be confirmed by immunoblotting using lysates of virus infected cells and probing with monoclonal **antibodies** specific for the 67 kDa antigen...

...taken from rats in groups II and III recognise the Theileria 67 kDa antigen on **Western** blots. Furthermore, the sera from these animals completely neutralise sporozoite infectivity in the in vitro...

...the presence of carbohydrate sidechains on the Theileria antigen are not essential for evoking neutralising **antibodies** .

20/3,KWIC/94 (Item 31 from file: 654)
DIALOG(R)File 654:US Pat.Full.
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3390293
Derwent Accession: 1992-325548
Utility
C/ **L-fucose dehydrogenase gene, microorganism having said gene and production of L-fucose dehydrogenase by the use of said microorganism ; CULTURE PRODUCT OF ESCHERICHIA**
Inventor: Mitta, Masanori, Tsuzuki, JP
Sakai, Takeshi, Hirosaki, JP
Kotani, Hirokazu, Moriyama, JP
Kato, Ikunoshin, Uji, JP
Assignee: Takara Shuzo Co., Ltd.(03), Kyoto, JP
Takara Shuzo Co Ltd JP (Code: 02633)
Examiner: Wax, Robert A. (Art Unit: 184)
Assistant Examiner: Hendricks, Keith D.
Law Firm: Wenderoth, Lind & Ponack

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5217880	A	19930608	US 92855793	19920323
Priority				JP 9189184	19910329

Fulltext Word Count: 4137

Description of the Invention:
...Next, the plasmid pTFDH101 was inserted into E. coli GM33 cells lacking **dam methylase** , and the recombinants were cultured, and plasmid DNA was prepared from them. This plasmid DNA...evaluate the extent of its expression. With this invention, it is also possible to produce **antibodies** based on the amino acid sequence of the enzyme...

20/3,KWIC/96 (Item 33 from file: 654)
DIALOG(R)File 654:US Pat.Full.
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3373082
Derwent Accession: 1988-161622
Utility
REASSIGNED
C/ **Method of producing bioadhesive protein ; MARINE ANIMAL EXTRACTS**
Inventor: Maugh, Kathy J., Walnut, CA
Anderson, David M., Rockville, MD
Strausberg, Robert, Silver Springs, MD
Strausberg, Susan L., Silver Springs, MD
Assignee: Enzon Labs Inc.(02), Gaithersburg, MD
Enzon Labs Inc (Code: 29105)
Examiner: Schwartz, Richard A. (Art Unit: 185)
Assistant Examiner: Mosher, Mary E.

Law Firm: Sterne, Kessler, Goldstein & Fox

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5202236	A	19930413	US 90528762	19900525
Division	Abandoned			US 8782456	19870807
CIP	Abandoned			US 86933945	19861124
CIP	Abandoned			US 84650128	19840913

Fulltext Word Count: 16894

Description of the Invention:
...Production of **Antibody** to Bioadhesive Precursor Protein...

...Booster subcutaneous injections using incomplete Freund's adjuvant were given subsequently in two-week intervals. **Antiserum** with high-titer **antibody** reactive toward the decapeptide as well as M. edulis bioadhesive precursor protein isolated from mussels...and pGX2346 are grown for DNA preparation in an E. coli host that contains the **dam** mutation (defective in DNA adenine **methylase**) so that they could be digested with BclI. The non-methylated pGX2346 DNA is cut...19. Production of the bioadhesive precursor protein is analyzed by **Western** Blot and SDS-polyacrylamide gel electrophoresis of the yeast proteins following procedures known in the...nm and by SDS polyacrylamide gel electrophoresis using both coomassie blue protein stain and the **Western** blot assay with specific . **antibodies** (Example 1). The fractions containing the bioadhesive precursor protein are pooled and dialyzed overnight twice...characterized by its mobility on SDS-polyacrylamide gel electrophoresis using both Coumassie blue stain and **Western** blot analysis, its UV absorption spectrum, protein quantitation and amino acid composition analysis...

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     $22.57  Estimated cost this search
     $22.57  Estimated total session cost   1.123 DialUnits

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